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A negative regulator of ribosomal RNA processing specifies R-gene-independent cell death in barley - powdery mildew interactions

Liu Xi
Iowa State University

A negative regulator of ribosomal RNA processing specifies *R*-gene-independent cell death in barley - powdery mildew interactions

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Liu Xi

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Roger Wise, Major Professor

Dan Nettleton

Steve Whitham

Iowa State University

Ames, Iowa

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ABSTRACT

Programmed Cell Death (PCD) plays a pivotal role in plant development and defense. Pathogen dependent cell-death mutants were used to investigate the complex regulatory pathways between PCD and *R*-gene mediated resistance. Time-course expression profiles of *Blumeria graminis* f. sp. *hordei* (*Bgh*) challenged C.I. 16151 (harboring the *Mla6* powdery mildew resistance allele) and its fast-neutron-derived "*Bgh*-induced tip cell death1" mutant, *bcd1*, were compared using the 22K Barley1 GeneChip. Contrasts were made to identify genes associated with the cell death phenotype as opposed to *R*-gene mediated resistance. One hundred eighty-two genes were found in the intersection of these contrasts at a threshold *p* value < 0.001 (equivalent false discovery rate < 5%). One hundred forty-seven of these 182 genes were found to be constitutively overexpressed in the *bcd1* mutant. GO annotation indicates that these genes are mainly involved in metabolism, showing common physiological process with other stressed-induced genes. Six deleted genes that co-segregated with the cell death phenotype mapped to the same region on chromosome 7 (5H), and are highly syntenous with rice. F_2 segregation analysis of crosses between genotypes harboring *bcd1* and *Mla6* × *Bcd1* and *mla6* demonstrated that the tip cell death was independent of *R*-gene mediated resistance. Virus induced gene silencing (VIGS) of one of the six deleted genes, CA031190, phenocopied *bcd1*-mediated tip cell death. These findings suggest that *Bcd1* mediates metabolism involved in cell death progression as a result of signaling during the barley-powdery mildew interaction, but is independent of gene-for-gene resistance.

CHAPTER 1. GENERAL INTRODUCTION

THESIS ORGANIZATION

This thesis describes the identification of a negative regulator of ribosomal RNA processing, which mediates an *R*-gene-independent cell death in barley. The literature review describes the plant host, pathogen and their relationship, cell death in plants and the major techniques used, including transcript-based cloning and Virus-Induced-Gene-Silencing. The paper “A Negative Regulator of Ribosomal RNA Processing Specifies *R*-gene-independent Cell Death in Barley - Powdery Mildew Interactions" is to be submitted to the journal of Plant Physiology. References are listed following both the literature review and the paper. A general conclusion follows the paper.

LITERATURE REVIEW

Barley

Barley (*Hordeum vulgare* L.) is an annual cereal grain with two growing seasons, winter and spring. It belongs to the grass family *Poaceae* and is descended from wild barley (*Hordeum spontaneum*) (von Bothmer and Jacobsen, 1985). As one of the oldest domesticated grain crops, it has been widely used in animal feed, human food, and brewing. It ranks fourth in the cereal production and in the area of crop cultivation worldwide (Poehlman, 1985). Being able to grow in a range of extreme environments, barley is well-known for its adaptability and tolerance to cold, drought, alkali, and salinity. In addition, its fast growth permits it to compete well with weeds and other grasses, making it an economical crop (http://barleyworld.org/index.php). Barley is a diploid with 7 pairs of chromosomes, containing approximately 5×10^9 bp DNA (Arumuganathan and Earle, 1991). It is self-fertile, easy to hybridize and grow. Moreover, the extensive natural variation makes it quite responsive to artificial selection. These attributes make it a favorable model for genetic studies of cereal crops. Molecular mapping of the barley genome projects have been initiated and facilitated by molecular marker development and genetic and bioinformatic resources (Kleinhofs and Han, 2002). With international research efforts, about 460,000 barley ESTs are now available in the GenBank (Hayes and Szucs, 2006). Growing genomic information has been available in public databases, such as BarleyBase in PLEXdb, a community resource for plant and plant-pathogen microarrays (Shen et al., 2005; Wise et al., 2007), HarvEST:Barley in HarvEST, a EST database-viewing software available at http://harvest.ucr.edu/, and barley germplasm database (http://www.shigen.nig.ac.jp/barley/).

Barley Powdery Mildew Resistance

Barley powdery mildew is a fungal disease caused by an ascomycete *Blumeria graminis* f. sp. *hordei* (*Bgh*). *Bgh* is an obligate biotroph, which can only survive on living barley plants. It only infects the epidermal cells; the mycelium on the surface of leaves resembles small cushion of white powder, and the disease become severe in wet and cool environment (Braun et al., 2002). In terms of interaction between plant and pathogen, barley and *Bgh* display standard gene-for-gene specificity. A large number of genes that confer resistance to *Bgh* have been identified in barley (designated as *Ml*) (Hinze et al., 1991; Giese et al., 1993; Görg et al., 1993; Jørgensen, 1994). Moreover, 32 *Mla* alleles on chromosome 5 (1H) have been found according to their specificities to distinct *Bgh* isolates (Giese, 1981; Wise and Ellingboe, 1985; Jørgensen, 1994; Kintzios, 1995). These, together with the distinctive phenotypes of different barley varieties inoculated with unique *Bgh* isolates make barley-powdery mildew an ideal system for investigating the interactions between plants and obligate pathogens (Wise and Ellingboe, 1985; Wei et al., 1999).

Plant Cell Death

Programmed cell death (PCD) is the death of cell carried out in a regulated process and serves essential roles in animal and plant development (Kerr et al., 1972; Ameisen, 1996; Vanyushin et al., 2004). In plants, PCD occurs in various developmental stages and during response to environmental stress and pathogen attack (Pennell and Lamb, 1997; Greenberg and Yao, 2004; Lam, 2004). In development, PCD can be classified by leaf senescence, root cap development, cell wall modification, gradual disappearance of organelles and cytoplasm, leaf shape sculpturing (Lam, 2004; van Doorn and Woltering, 2005). When plants are exposed to an unfavorable condition, PCD can be induced.

Examples are waterlogging, hypoxia, mechanical block, and nutrient deficiencies (Hofius et al., 2007). Abiotic factors, such as ozone, heat shock and ultraviolet (UV) irradiation, can also trigger cell death as indicated by the oxidative burst (Rao et al., 2000; Danon et al., 2004; Vacca et al., 2004). In plant-pathogen interactions, when a pathogen successfully infects the plant, cell death can facilitate the disease development; while cell death occurring in incompatible interactions triggers localized host cell death, restricting pathogen invasion (Gilchrist, 1998; Greenberg and Yao, 2004).

Genes that have distinctive expression patterns in various PCDs suggest different signaling pathways among them. For example, *HSR203J,* which is specific to hypersensitive response, or *SAG12* which was only detected during senescence (Pontier et al., 1999). However, despite variety of cell death forms, common steps exist among different PCDs. Several studies have shown the overlap of defense-related and senescence-induced genes (Quirino et al., 1999; Quirino et al., 2000). For example, the tobacco *HIN1* gene, an HR cell death marker, was found to express at late stages of leaf senescence (Takahashi et al., 2004).

When attacked by pathogens, plants can develop cell death in either incompatible or compatible interactions. The former one is known as hypersensitive response (HR) and the latter one can be necrosis development (Greenberg and Yao, 2004). Although the following disease development in these two situation are opposite, studies have shown that both HR-related cell death and necrosis shared the same features including chromatin condensation and DNA fragmentation (Mittler et al., 1996; Ryerson and Heath, 1996; Wang et al., 1996). The HR typically occurs in a gene-for-gene situation, in which the resistance is controlled by resistance (*R*) genes in the plant recognizing corresponding avirulence (*Avr*) genes in the pathogen (Flor, 1971). A good example is the *Pto* gene, which confer resistance to bacterial speck disease in tomato by eliciting hypersensitive response (Zhou et al., 1995; Zhou et al.,

1997). However, several studies have reported the separation of defense gene activation from cell death. For instance, the lesion mimic *lsd* mutant of *Arabidpopsis* showed reduced PR gene expression and resistance to *Peronospora parasitica* while still retaining cell death (Hunt et al., 1997). In oat, *Rds* was showed to suppress HR without affecting resistance while *Rih* confers cell death in both resistant and susceptible plants (Yu et al., 2001). These results suggest that signaling pathways that lead to defense and cell death could become separate while overlap at certain points. However, as for the questions of how and where this crossover and separation happen remain to be answered.

Transcript-based Cloning

Microarray technology provides an efficient way for parallel analysis of gene expression in various biological process (Zhu, 2003). It makes possible the parallel comparison of expression of thousands of genes in a single experiment and systematic assessment can be made for a particular biological process (Close et al., 2004). GeneChip is a specialized microarray manufactured by Affymetrix (http://www.affymetrix.com). It uses *in-situ* synthesized DNA oligonucleotides as probes to detect the sequence similarity and abundance of target nucleotide molecules through complementary-sequence binding (Lipshultz, 1999). The derived expression profiling is then analyzed using standard data-processing methods, which facilitate the data mining through a normalized database. At the same time, because of their extensive coverage, GeneChip has been broadly used in genome-wide expression analysis (Zhu, 2003). A number of GeneChips have been developed for plants, including *Arabidopsis* (Zhu and Wang, 2000), maize (Hunter et al., 2002), rice (Zhu et al., 2003), barley (Close et al., 2004), medicago, and wheat. In this study, we used the 22K Barley1 GeneChip as the beginning platform to analyze the transcript profiles of wild-type versus mutant plants upon pathogen attack.

Recently, transcript-based cloning has emerged as alternative method to the traditional positional cloning to isolate phenotype generating genetic elements (Mitra et al., 2004). It is a gene cloning approach based on the transcripts polymorphism between wild-type and mutant. It is easy to identify the mutated genes according to the significant difference in expression comparison between the wild-type and mutants, especially those mutants with deletions, such as the fast-neutron derived mutants (Zakhrabekova et al., 2002; Zhang et al., 2006; Wise et al., 2007). Several studies have shown the potential of transcript-based cloning in the rapid cloning of important genes in both model and crop plants. Examples are the cloning of *DMI3*, a Ca^{2+}/calmodulin-dependent protein kinase of *Medicago* (Mitra et al., 2004), and the identification of a region containing *Rpr1*, a gene required for *Rpg1*-dependent resistance to stem rust in barley (Zhang et al., 2006). Candidate genes identified through transcript-based cloning can be further characterized by cosegregation analysis and functional analysis, such as gene silencing and overexpression, and then subject to transformation, allelic mutant analysis, or transient assays (Wise et al., 2007).

Virus Induced Gene Silencing

Post-transcriptional gene silencing (PTGS), or RNA silencing, is a process in which the expression of an gene is inhibited based on its homology to an introduced gene (Napoli et al., 1990; van der Krol et al., 1990; Depicker and Montagu, 1997; Stam et al., 1997). In general, double strand (ds) RNA can be recognized and cut by an enzyme called Dicer. This generates small interfering (si) RNA with about 21-25 nucleotides. These siRNA are incorporated into an RNA-induced silencing complex (RISC), which then directs the degradation of target mRNA. In the meantime, the original siRNAs can be amplified by an RNA-dependent-RNA polymerase (RdRp), creating more siRNAs. This process augments

the population of siRNA and RISC, facilitating the RNA degradation (Depicker and Montagu, 1997; Hutvagner et al., 2001; Ibrahim et al., 2006; Macrae et al., 2006; Lehmann et al., 2007). Specifically, when a plant is infected with a virus, the replication of the virus makes double strand intermediates, which then can be recognized by Dicer and activates the RNA silencing to target the invasive RNA (Lu et al., 2003).

Based on the RNA-mediated defense mechanism described above, virus- induced gene silencing (VIGS) was developed as an efficient approach used in plant functional genomics. In this method, a fragment from the host gene is inserted into a virus vector, which is then used to infect plants. As described before, the viral dsRNA replication intermediates are processed and generates siRNAs corresponding to the viral vector genome, including the insert. Therefore, the RISC complex would target the matching host mRNA, leading to the failure of subsequent protein synthesis or function (Lu et al., 2003). This approach is currently being successfully applied in plants and used extensively in gene function analysis and modified to high-throughput functional genomics (Holzberg et al., 2002; Liu et al., 2002; Burch-Smith et al., 2006; Ding et al., 2007).

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CHAPTER 2. A NEGATIVE REGULTOR OF RIBOSOMAL RNA PROCESSING SPECIFIES *R*-GENE-INDEPENDENT CELL DEATH IN BARLEY – POWDERY MILDEW INTERACTIONS

A paper to be submitted to the journal of Plant Physiology

Liu Xi[1,1], Matthew J. Moscou[1,2], Rico A. Caldo[1*], Yan Meng[1], Dan Nettleton[3], Roger P. Wise[1,2,4,2]

1 Department of Plant Pathology and Center for Plant Responses to Environmental Stresses, Iowa State University, Ames, IA, 50011-1020, USA

2 Bioinformatics and Computational Biology, Iowa State University, Ames, IA 50011-3260, USA

3 Department of Statistics, Iowa State University, Ames, IA, 50011-1210, USA

4 Corn Insects and Crop Genetic Research, USDA-ARS, Iowa State University, Ames, IA, 50011-1020, USA

***Current address:** Monsanto Company, St. Louis, MO, 63167, USA

ABSTRACT

Programmed cell death (PCD) plays pivotal roles in plant development and defense. Pathogen dependent cell-death mutants were used to investigate the complex regulatory pathways between PCD and *R*-gene mediated resistance. Time-course expression profiles of *Blumeria graminis* f. sp. *hordei* (*Bgh*) challenged barley cultivar C.I. 16151 (harboring the

[1] Primary researcher and author
[2] Author for correspondence

Mla6 powdery mildew resistance allele) and its fast-neutron-derived "*Bgh*-induced tip cell death1" mutant, *bcd1*, were compared using the 22K Barley1 GeneChip. Contrasts were made to identify genes associated with the cell death phenotype as opposed to *R*-gene mediated resistance. One hundred eighty-two genes were found in the intersection of these contrasts at a threshold p value < 0.001 (FDR < 5%). One hundred forty-seven of these 182 genes were found to be constitutively overexpressed in the *bcd1* mutant. GO annotation indicates that these genes are mainly involved in metabolism, showing common physiological process with other stressed-induced genes. Six deleted genes that co-segregated with the cell death phenotype mapped to the same region on chromosome 7 (5H), and are highly syntenous with rice. F_2 segregation analysis of crosses between genotypes harboring *bcd1* and *Mla6* × *Bcd1* and *mla6* demonstrated that the tip cell death was independent of *R*-gene mediated resistance. Virus induced gene silencing (VIGS) of one of the six deleted genes, CA031190, phenocopied *bcd1*-mediated tip cell death. These findings suggest that *Bcd1* mediates metabolism involved in cell death progression as a result of signaling during the barley-powdery mildew interaction, but is independent of gene-for-gene resistance.

INTRODUCTION

Programmed Cell Death (PCD) plays an essential role in plants and is regulated by various pathways. Examples are the initiation and execution of senescence during plant development, the hypersensitive response (HR) during pathogen attack, as well as that which occurs under various abiotic stresses (Lam, 2004). PCD can be initiated by internal or external factors and is under control by active genetic programs. During leaf senescence, cell death usually starts from the tips or margins and progresses towards the base; however, earlier senescence can occur on a targeted part of the plant under environmental stress

(Lim et al., 2007). HR is triggered when the plant recognizes a pathogen and generates a local cellular suicide halting pathogen progression (Sasabe et al., 2000). Common and distinct steps exist among different PCDs. Several studies have shown the overlap of defense-related and senescence-induced genes (Quirino et al., 1999; Quirino et al., 2000). For example, the tobacco *HIN1* gene, an HR cell death marker, was found to express at late stages of leaf senescence (Takahashi et al., 2004). Also, several genes have been found to have distinct expression patterns, such as *HSR203J,* which is specific to HR or *SAG12,* which was only detected during senescence (Pontier et al., 1999). However, the crossover and dissection of these different pathways remain to be elucidated.

During plant-pathogen interactions, cell death can occur in both incompatible and compatible responses. It is normally believed that, in the incompatible response, HR is activated by the R-AVR interaction, which restricts the pathogen ingress; whereas, compatibility caused cell death may facilitate the pathogen infection (Kim and Palukaitis, 1997; Greenberg et al., 2000). However, recent studies assert that cell death may be a consequence, rather than a cause during plant-pathogen interactions. For instance, the *Rx*-gene conditions resistance to *Potato virus X* (PVX) without cell death, but the PVX coat protein elicits cell death when transformed into the *Rx* plants, leading to the hypersensitive response (Bendahmane et al., 1999). Evidence in oat has shown *Rds* and *Rih* mediated HR were independent of gene-for-gene resistance to oat crown rust (*Puccinia coronata* f. sp. *avenae*) (Yu et al., 2001). *Arabidopsis dnd1* mutant retains gene-for-gene resistance in defense response with the reduced ability to produce HR (Yu et al., 1998; Yu et al., 2000). Therefore, diverse cell death types occur in various pathways and play different roles during plant-pathogen interactions.

The cellular features of cell death can be characterized by fragmentation of nuclear DNA, signal transduction involving Ca^{2+} fluxes, changes in protein phosphorylation, increase

in nuclear heterochromatin and induction of reactive oxygen species (Greenberg, 1996; Pennell and Lamb, 1997). In plants, cell death is often correlated with development and metabolism, and their associated differentiation, reproduction and vegetative growth (Beers, 1997; Tamagnone et al., 1998). The exosome complex plays an essential role in RNA metabolism, mediating 3'-->5' RNA processing and degradation (Mitchell et al., 1997; Estevez et al., 2003; Chekanova et al., 2007). Allmang et al. (2000) reported the functions of the exosome in rRNA, snoRNA and snRNA synthesis. Chekanova et al. (2007) used genome-wide high-resolution mapping of exosome substrates to demonstrate the regulatory role of the exosome in mediating RNA quality control and stable structural RNA metabolism in plants. Recent studies showed that the exosome has cell death related nuclease function, leading to apoptotic DNA degradation in *C. elegans* and mammals (Parrish and Xue, 2006), but little is reported concerning the role of the exosome during plant-pathogen interactions.

Barley (*Hordeum vulgare*) powdery mildew, caused by *Blumeria graminis* f. sp. *hordei (Bgh)*, is an ideal system to study the interactions between obligate fungal biotrophs with their hosts (Caldo et al., 2004). The well-characterized *Bgh* infection stages (Clark et al., 1993; Hall, 1999), and indistinguishable fungal development in the early infection stages during compatible and incompatible interactions (Boyd, 1995) provide the opportunity to monitor the host response upon pathogen attack. In this study, we identified the fast-neutron-derived cell death mutant *bcd1* and used the 22K Barley1 GeneChip to compare transcript profiles of wild-type versus mutant upon pathogen attack. Bioinformatic and functional analytic methods were used to elucidate that *bcd1*, encoding a ribosomal RNA (rRNA) processing protein, mediates an *R*-gene-independent cell death. Our results suggest a role of rRNA processing genes in mediating defense, implying a common cell death pathway existing besides the compatible and incompatible interface upon pathogen infections.

RESULTS

Mutant Selection and Experimental Design

The study of mutants involved in pathogen-induced cell death is an efficient way to unravel the regulation underlying different genes that function in the crosstalk and direction of cell-death-mediated pathways (Lorrain et al., 2003). In host-pathogen interactions, the variety of host responses to pathogen attack offers the way to answer specific questions by selecting proper plant-pathogen combinations (Caldo et al., 2004). Since cell death can occur in both compatible and incompatible interactions, we are interested in comparing the time-course expression profiles of wild-type with mutant in both situations. Here we utilized a tip cell death mutant *bcd1* selected from a group of fast-neutron-derived C.I. 16151 mutants, where *bcd1* was recognized by developing tip cell death upon *Bgh* inoculation while retaining resistant or susceptible response to incompatible or compatible isolates, respectively (Figure 1).

As shown in Figure 1, our experimental design contains the wild-type C.I. 16151 (*Mla6*) and its fast-neutron-derived mutant *bcd1*, challenged with the *Bgh* isolate 5874 (AVR_{a6}) and K1 (avr_{a6}), respectively. The experiment was conducted based on a split-split-plot design described by (Caldo et al., 2004) with replications as blocks, *Bgh* isolate as the whole-plot factor, plant genotype as the split-plot factor, and time as the split-split-plot factor. Fifteen first leaves of inoculated barley seedlings were harvested at 0, 8, 16, 20, 24, and 32 hours after inoculation (hai). One Barley1 GeneChip (Close et al., 2004) was used for each of the 72 split-split-plot experiment units corresponding to 3 replications x 2 isolates x 2 genotypes x 6 time points.

Transcript Profiling Analysis

The statistical analysis used in this study was based on the overall pattern of expression, reflecting the kinetics of the plants response to pathogen infection (Caldo et al., 2004). Our primary analysis strategy was to identify genes that were differentially expressed between wild-type and mutant over time. In order to eliminate genes related to isolate effects, three contrasts were made to group genes associated with the cell death phenotype as opposed to *R*-gene-mediated resistance. The first contrast compared plants of the C.I. 16151 line (containing *Mla6*) to its fast-neutron- derived mutant *bcd1*, both challenged with *Bgh* isolate 5874 (AVR_{a6}) over time. This comparison yielded those genes differentiable between C.I. 16151 and *bcd1* in response to the *Bgh* isolate 5874. Similarly, the second contrast was made in response to *Bgh* isolate K1 (avr_{a6}). The third contrast took an average of the expression for each genotype in response to the two isolates before contrasting C.I. 16151 with *bcd1*. These contrasts identified genes with small genotype x isolate but large genotype x time effects. Thus, we can compare the difference between these two genotypes throughout the time course.

Based on the analysis strategy described above, 182 genes were found to represent the intersection of these contrasts with a cutoff $p < 0.001$, with a false discovery rate (FDR) of 5% (Storey and Tibshirani, 2003). Excepting 6 genes with variable patterns, 147 genes out of these 182 were found to be constitutively overexpressed in the mutant compared to the wild-type; while 29 genes had the constitutively lower expression than the wild-type (Figure 2). To understand the biological interpretations of these differentially expressed genes, Gene Ontology (GO) – based functional enrichment analysis was performed by using EasyGO (Zhou and Su, 2007). In the “biological process” category, genes fall into different stages of cellular or metabolic processes, (Figure 3a), and the metabolic processes involving these genes is very similar to that of a group of cold-induced genes in

Arabidoposis (Yamaguchi-Shinozaki and Shinozaki, 2005; Zhou and Su, 2007). When GO terms were assessed for "cellular component", most of these genes contribute to the ribosome biosynthesis, involving cell parts, organelles and the macromolecular complex. This is consistent with the molecular function of these genes, most of which are the structural constituents of ribosome (Figure 3b, c).

It is known that fast neutron can cause deletions (Li et al., 2001), thus, the expression change in *bcd1* could be caused by deletion of certain regulator(s), which regulate a co-expressed cluster of genes associated with the leaf tip cell-death. Twenty-two candidate genes were identified by probe sets that had > 4.5 fold-change between wild-type and mutant in addition to the expression level in *bcd1* of < 3 (natural log value) through all 6 time points (Supplemental Table 2). Model Genome Interrogator at PLEXdb (http://plexdb.org/) allows a user to input a selected list of genes from a plant GeneChip to derive the physical position and sequences on the Arabidopsis or rice genomes. This tool was used to position the twenty-two Barley1 probe sets on rice chromosomes 1, 2, 3, 5, with hyperlinks to the alignments on the Gramene rice genome browser (http://www.gramene.org/).

Six Deleted Genes Co-segregate with *R*-gene Independent, *bcd1*-Mediated Tip Cell Death

Deletion gene candidates derived from the microarray analysis were tested by genomic PCR and RT-PCR. Five out of the 22 genes represented by Barley1 probesets Contig12722_s_at, Contig24342_at, Contig8225_at, Contig4201_s_at, and Contig9277_s_at, were found to be deleted in the *bcd1* mutant (Table 1, Figure 4). Two sets of genetic crosses were performed to test whether 1) the deleted genes cosegregated with the *bcd1* phenotype, and 2) the cell death is independent of *R*-gene mediated

resistance. Specifically, the *bcd1* mutant (*Mla6/Mla6, bcd1/bcd1*) was crossed to (1) wild-type C.I. 16151 (*Mla6/Mla6, Bcd1/Bcd1*) and (2) cv. Morex (*mla/mla, Bcd1/Bcd1*), respectively. Seven-day old F_2 seedlings were inoculated with *Bgh* 5874 and phenotyped another 7 days after inoculation. As for the first cross, wild-type vs. plants displaying the tip cell death phenotype was 42 and 18, respectively, which fit the expected 3:1 ratio (Table 2). In the second test, twenty-two plants displayed resistance without cell death, 3 plants showed resistance and cell death, 7 plants exhibited susceptibility without cell death and 3 plants presented susceptibility and cell death, fitting a 9:3:3:1 ratio (Table 2). DNA was extracted from each plant of the populations and used as template in PCR to amplify the 5 probe sets (Contig12722_s_at, Contig24342_at, Contig8225_at, Contig4201_s_at, and Contig9277_s_at). In both populations, except for Contig9277_s_at, no PCR products for all other 6 genes were amplified from DNA of those plants with cell death phenotype compared to those without cell death (Figure 5). This indicates that gene Contig12722_s_at, Contig24342_at, Contig8225_at, and Contig4201_s_at co-segregated with *bcd1*-mediated cell death. According to the result derived from the Model Genome Interrogator described before, these four genes all mapped to the same region of rice chromosome 3 while gene Contig9277_s_at mapped to chromosome 5. In addition, four neighboring genes of the deletion region were identified using rice synteny at GRAMENE and subjected to the PCR tests as before, and two of them were found to be deleted (CA031190 and BF267800) and co-segregated with *bcd1* cell death (Figures 4 and 5). Therefore, 4 genes represented by Barley1 probesets Contig12722_s_at, Contig24342_at, Contig8225_at, Contig4201_s_at and 2 genes derived from rice sytenic region (Genbank ID CA031190 and BF267800) were found to be deleted in *bcd1* mutant and co-segregated the tip cell death phenotype. At the same time, results from the second crosses also demonstrated that these 6 genes in the

deletion were independent of the *R*-gene, as with the homozygous deletion plants cosegregated with cell death regardless of resistant and susceptible responses (Figure 5b).

Genetic and Rice Synteny Mapping Positions the Deleted Region on Chromosome 7 (5H)

We utilized expression quantitative trait loci (eQTL) and transcript-derived marker (TDM) data from an expression profiling experiment using the Steptoe x Morex (SxM) doubled haploid mapping population (Potokina et al., 2008) to genetically map the deleted probe sets to chromosome 7 (5H). The region encompassing the *bcd1* deletion is highly syntenous with rice, and the barley probe sets identified (using tBlastn) from rice synteny are ordered correctly within this region based on the TDM and eQTL data (Figure 4). The proximal (left) border of the deletion is gene-rich and permits a clear delineation of the breakpoint between Contig8931_at (LOC_Os03g63710) and CA031190 (LOC_Os03g63720). The distal (right) border is extremely gene-poor, where most annotations of rice genes are retrotransposons of the classes Ty1-copia, Ty3-gypsy, a novel protein containing a WRKY domain, and unclassified genes. The closest proximal gene which could be identified to the deletion breakpoint was Contig10531_at (LOC_Os03g63860), and was found to be present and expressed (Figure 4). Thus, we could predict the size of the deletion in rice to be between 30 to 70 kb, although barley is expected to have a much larger size due to repetitive elements.

Silencing of *Bcd1* Causes Tip Cell Death in Barley Leaves

BSMV-mediated virus-induced gene silencing (BSMV-VIGS) was used to assess the functions of the six genes contained within the co-segregating deletion. Two pairs of primers were used to amplify fragments for each of the 6 deleted genes from cDNA of wild-

type C.I. 16151 (Table 3). VIGS constructs containing above individual cDNA insert were made according to the method of Holzberg et al. (2002). Seven-day-old C.I. 16151 plants were bombarded with each VIGS construct. Yellow-and-white stripe viral symptoms were observed in BSMV-infected leaves as the plants developed (Figure 6a). Infected leaves were collected and grounded to obtain recombinant virions, which were used to mechanically infect 7-day-old C.I. 16151 plants with 8 seedlings in each treatment. After 14 days, plants were inoculated with *Bgh* 5874 and phenotyped after an additional 7 days. Optimal silencing occurred in the third leaf stage, and was used for phenotyping (data not shown). Plants infected with BSMV:CA031190_1 or BSMV: CA031190_2 developed tip cell death equivalent to the *bcd1* mutant. No cell death was observed in the empty vector (BSMV:00) and mock, as well as other constructs (Figure 6a). The whole experiment was repeated four times with each replicate producing similar results. Semi-quantitative RT-PCR showed that CA031190 was silenced in the plants with the cell death phenotype as indicated by the reduction of transcripts of fragments in CA031190 compared to the Actin internal control (Figure 6b). These results indicate that CA031190 represents the *Bcd1* gene that mediates the tip cell death.

***Bcd1* is Similar to Proteins with a Ribosomal RNA Processing Function**

Sequence analysis of CA031190 using MacVector revealed that the 656 bp EST contains a partial open reading frame (ORF) of 654bp, encoding a peptide of 218 amino acids. A protein domain search was done using Pfam 22.0 (Finn et al., 2006), revealing that the protein has 49.5% similarity to RNase-PH, which contains domain 1 of a 3' exoribonuclease family. In the peptide, the 3' exoribonuclease domain 1 starts from position 11 to 131, consisting of 121 amino acids. A Blastp search of this protein in TIGR rice genome annotation (http://www.tigr.org/tdb/e2k1/osa1/) revealed that the putative function of

this protein is the exosome complex exonuclease RRP46 (Ribosomal RNA-processing protein 46), which is a protein involved in rRNA processing and a component of the exosome 3'-->5' exonuclease complex (Allmang et al., 1999a). A cross-taxa alignment of 11 RRP46 annotated sequences from plants (*H. vulgare, Oryza sativa, Arabidopsis thaliana*), vertebrates (*Homo sapiens, Mus musculus*), nematodes (*Caenorhabditis elegans*), fungi (*Saccharomyces cerevisia, Schizosaccharomyces pombe, Neurospora crassa*), insects (*Anopheles gambiae, Drosophila melanogaste*r) was conducted using the Multiple sequence alignment (Corpet, 1988). As shown in Figure 7, the putative barley RRP46 is broadly conserved with other RRP46 proteins among phylogenetically diverse taxa.

As indicated by GO analysis, the 182 differentially expressed genes are localized in or associated with the ribosome group (Figure 3b). Annotation of these genes showed that most of them encode either components of the ribosome or are involved in the anabolism of ribosomal elements. Moreover, in the biological process, these genes operate in different physiological and cellular processes (Figure 3a), where they represent structural molecule activity at the molecular level, making structural constituent of ribosome (Figure 3c). Recent studies showed that some ribosomal genes are involved in plant defense and plant cell death (Quirino et al., 1999; Chekanova et al., 2000; Stirpe and Battelli, 2006). It is possible that the deletion of *Bcd1* causes the failure of correct rRNA processing, causing the cell to produce excessive coordinating ribosomal protein. Therefore, the abundance of ribosomal RNA and protein alter the plants' metabolism, impairing plants' defense system and facilitating the cell death development upon pathogen attack.

DISCUSSION

Deletion of *Bcd1* Preconditions Barley Tip Cell Death

Pathways for various forms of cell death have been found to have distinct and overlapping levels of interaction. Regardless of the path taken, the final destination is the suicide of plant cell. Unlike lesion-mimic mutants that can automatically develop cell death, the *bcd1* mutant was found to exhibit a tip cell death after *Bgh* inoculation; silencing of *Bcd1* generates an equivalent phenotype. This indicates that the absence of *Bcd1* preconditions the tip cell death development. It is possible that plants' normal physiological or defense process is compromised by losing *Bcd1*, making plants more vulnerable. Thus, when inoculated with *Bgh,* the pathogen generates an strong stress, which facilitates the collapse of plant cell, leading to accelerated leaf senescence, as senescence usually begins from the tip or margins (Lim et al., 2007). A simple explanation of accelerated senescence does not account for the accompanying necrosis, which indicates the underlying plant-pathogen interactions and associated signal transduction. Thus, the absence of *Bcd1* regulation may also lower the threshold for cell death in pathogen-attacked or pre-senescing cells (Shirasu and Schulze-Lefert, 2000).

Although pathogen-derived elicitors, such as microbe-associated molecular patterns (MAMPs) can trigger plant defense response (Navarro et al., 2004; Zipfel et al., 2004; Kim et al., 2005), tip cell death only occurs when the *bcd1* mutant is inoculated with viable *Bgh*. Thus, in this case, pathogen attack is required to induce cell death. Usually, during plant-pathogen interactions, cell death is believed to be directly associated with *R*-gene mediated defense, such as the hypersensitive response restricting the pathogens invasion while the necrosis of host tissues assisting in the pathogens invasion in compatible interactions (Morel and Dangl, 1997; Greenberg and Yao, 2004). Studies have shown that early *Bgh* recognition by the host, conidiospore adhesion, germination, and development of

appressoria are not fundamentally different in incompatible and compatible barley - *Bgh* interactions (Boyd, 1995). Since *bcd1* cell death occurs in both incompatible and compatible responses (Figure 1), it is possible that the initiation of cell death may occur during common pathogen infection stages, where timed regulation consists of early or late induction during barley-powdery mildew interaction. Further studies can be done to define the exact stage that cell death is induced. However, our results suggest that *Bcd1* is involved in plant-pathogen interactions and may function in plant defense. Therefore, the *Bcd1* that we have isolated appears to mediate an *R*-gene-independent cell death, and without *Bcd1*, plant succumbs to the stress response generated by *Bgh*.

Destabilization of the Ribosomal RNA/protein Signaling Pathway

Ribosomal RNA, the catalytic component of the ribosomes, accounts for approximately 80% of the total RNA in eukaryotic cell (Kampers et al., 1996). The primary transcripts produced from most rRNA genes are extensively processed to yield the mature, functional forms (Lodish et al., 2000). The coordinated rRNAs and ribosomal proteins (r-proteins) make the ribosome, translating RNA into protein. The proper amount and function of the ribosome ensures normal metabolism in plants. In this report, transcript profiling analysis revealed an abundance of ribosome constituent genes constitutively overexpressed in the *bcd1* mutant as compared to its wild-type parent throughout the 0 to 32 hour time course (Supplemental Table 1, Figure 2, 3). Therefore, we presume that the deletion of a particular regulator causes the failure of correct processing of pre-rRNAs, generating excessive rRNAs, to which the cell has to produce over-abundance corresponding r-proteins as opposed to its steady state requirement. This destabilizes the ribosomal RNA/protein signaling pathway, compromising the plants' normal metabolism and physiological process, thus, the plant becomes defenseless and prone to develop cell death under stress.

Dual Functions of *Bcd1* Leads to the Tip Cell Death

The exosome is a conserved multiexonuclease complex that mediates RNA processing and degradation (Mitchell et al., 1997). The exosome core is featured by the hexameric ring composed of six RNase PH domain-type proteins (RRP41, RRP42, RRP43, RRP45, RRP46 and MTR3) (Mian, 1997). In yeast and human, studies have shown that all the core subunits are vital to the normal activity of exosome to ensure its function (Allmang et al., 1999a; Allmang et al., 1999b; Liu et al., 2006). Recently, Parrish and Xue (2003; 2006) reported DNA degradation complex "degradeosome" in apoptotic DNA degradation in *C. elegans*. One of its components CRN-5 appears to be homologous to RRP46. Silencing of *crn-5* generates accumulation of TUNEL-reactive DNA intermediates in apoptotic cells, indicating its importance in apoptosis (Parrish and Xue, 2003). In our study, silencing of *Bcd1* causes tip cell death, suggesting its possible role in apoptosis that directly leads to the phenotype. On the other hand, the abundance of constitutively overexpressed ribosomal genes observed in *bcd1* mutant is consistent with the RNA processing function of RRP46. Thus, it is likely that without *Bcd1*, the superfluous rRNA and ribosomal protein compromise plants' normal metabolism and impairs the defense system, making them more susceptible to pressure and easily to develop cell death upon pathogen infection. Since the in *bcd1*-mediated cell death was coincident with constitutive expression of ribosomal related genes, we hypothesize that the *bcd1*-mediated cell death can be caused either by a *Bcd1* apoptosis function or by a compromised metabolism and defense due to loss-of-regulation of rRNA / r-protein processing, or by the combined effects of both.

MATERIALS AND METHODS

Fungal Isolates

Blumeria graminis f. sp *hordei* isolates 5874 (AVR_{a6}) and K1 (avr_{a6}) were propagated on *Hordeum vulgare* cv. Manchuria (C.I. 2330) in separate growth chambers at 18 °C with 16 hours light and 8-hours darkness.

Isolation of Fast-Neuton Derived Mutants

The C.I. 16151 line was obtained by introgression of the *Mla6* gene into the universal susceptible cultivar Manchuria (Moseman, 1972). Seeds of C.I. 16151 were treated with fast neutrons at 4 Gy Nf at the International Atomic Energy Agency, Vienna, Austria. M_1 seeds were space planted at the USDA-ARS Small Grains Laboratory in Aberdeen, Idaho. Single spikes from each individual M_1 plant were harvested to represent the M_2 family, which was screened for mutant segregates by sowing intact spikes consisting of 25-40 seeds in potting soil following the method of (Wise and Ellingboe, 1985). Each of 40 M_2 families as well as the susceptible control (cv. Manchuria, C.I. 2330) were sown per flat. When the first leaves were completely unfolded (~10 cm high), plants were inoculated with *Bgh* isolate 5874 (AVR_{a6}) and families were scored for infection type 7 days after inoculation. Seedlings that produced cell death symptoms or sporulating *Bgh* colonies were selected for rescue. Putative mutants deemed as homozygous by the 1 mutant : 3 wild-type segregation ratio were advanced to the M_3 generation, and then retested with *Bgh* 5874.

Experiment Design

Planting, stage of seedlings, harvesting, and experiment design were part of a larger experiment described by Caldo et al. (2004). Briefly, C.I. 16151 and *bcd1* were planted in separate 20 × 30-cm flats using sterilized potting soil. Each experimental flat consisted of

six rows of 15 seedlings, with rows randomly assigned to one of six harvest time points (0, 8, 16, 20, 24, and 32 hai). Seedlings grown to the 1st leaf stage with 2nd leaf unfolded were inoculated with a high density of fresh conidiospores (84 ± 19 spores/mm^2). Groups of flats were placed at 18 °C (8-hour darkness, 16-hour light) in separate controlled growth chambers corresponding to the *Bgh* isolates. Rows of plants were harvested at each assigned time points and snap frozen in liquid nitrogen. The entire experiment was repeated three times in a standard split-split-plot design with 72 experimental units (Kuehl, 2000).

Microarray Analysis

Total RNA was isolated using a hot (60 °C) phenol/guanidine thiocyanate method as described by Caldo et al. (2004). Probe synthesis, labeling and GeneChip hybridization, washing, staining, and scanning were performed the Iowa State University GeneChip Core facility. Data processing and normalization were performed according to Caldo et al. (2004). A mixed linear model (Wolfinger et al., 2001) was employed to analyze the 22,792 probe sets on the Barley1 GeneChip using the SAS MIXED procedure. Contrast statements as described before were used to identify genes signifying difference in average expression between wild-type and mutant upon pathogen challenge. For each contrast, time-specific differences between the average expressions were tested for equality using an *F*-statistic, and genes with this difference varied significantly (p-value < 0.001) across time points were identified as differentially expressed. FDR were measured to be < 5% at this threshold using the method described by Story and Tibshirani (2003). Gene Ontology enrichment depiction was done using the EasyGO tool located at http://bioinformatics.cau.edu.cn/easygo/.

Cluster analysis was carried out according to Caldo *et al*. (2004). In brief, average scaled signal intensities were calculated from three replications using Microsoft Excel. Data matrices were constructed with genes in rows and time points of the different genotype in columns. Hierarchical clustering of the 182 genes (Figure 2) was made using GeneSpring 5.1 (Silicon Genetics, Redwood City, CA) software, and Person correlation was used to calculate the similarities.

Data Access

Detailed data are publicly available at BarleyBase / PLEXdb (http://www.plexdb.org) under accession number BB46.

Genomic-PCR and RT-PCR

Leaves of 0 hour time point plant in the microarray experiment were used for RNA extraction (as described previously) of C.I. 16151 and the *bcd1* mutant. For RT-PCR, single-strand cDNA was synthesized using SuperScript™ III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) using oligo (dT) as a primer. PCR was performed subsequently using *Taq* DNA polymerase, recombinant (Invitrogen, Carlsbad, CA, USA) with specific primers according to each contig sequence (Table 1). PCR was also carried out with 1µg genomic DNA of the wild-type and the mutant, respectively, using the same sets of primers. Border genes (Contig8931_at and Contig10531_at) were used as positive controls (Figure 4, Supplemental Table 2).

Cosegregation Analysis

bcd1 (*Mla6, bcd1*) was crossed to the wild-type C.I. 16151 (*Mla6, Bcd1*) and Morex (*mla6, Bcd1*), respectively. Seven-day-old F_2 seedlings were inoculated with *Bgh* 5874.

Infection phenotypes were scored 7-days after inoculation. A single leaf from each seedling was harvested for DNA extraction. PCR was performed using the population DNA as the template and primers representing the deletion gene candidates (Supplemental Table 2). DNAs of C.I. 16151, *bcd1,* Morex, and Manchuria were used as controls.

BSMV Constructs

VIGS constructs were used to silence candidate genes in wild-type C.I. 16151. BSMV, a tripartite RNA virus consisting of α, β and γ genomes, was used as the silencing vector. The procedure was following the method of Holzberg et al. (2002). Briefly, PCR was performed to amplify fragment of candidate genes from cDNA of C.I. 16151 using the primers containing *Not*I and *Pac*I restriction sites at each end (Table 3). Each amplified fragment was digested with *Not*I and *Pac*I and inserted in antisense orientation into the γ vector to make BSMV silencing constructs.

Semi-quantitative RT-PCR

The first strand cDNA was generated from RNA extracted from plants in VIGS experiments using an oligo (dT) primer and reverse trascriptase as described before. This first strand cDNA was used in PCR reactions using gene specific primers (Table 4) at 25, 30, 35, 40 cycles, respectively. Actin was used as an internal control.

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Table 1. Predicted Functions of 7 Deleted Genes in *bcd1* Mutant

Affymetrix Probe Set ID	GenBank ID[a]	Unigene ID[b]	Predicted Function[c]
	CA031190[d]	15423	3' exoribonuclease family, domain 1 containing protein, expressed
Contig12722_s_at	BG415383	12722	RNA recognition motif, putative, expressed
Contig24342_at		24342	Expressed protein
Contig8225_at	BQ762325	8225	Heat shock factor protein HSF8, putative, expressed
	BF267800[d]	39836	Xyloglucan endotransglucosylase/hydrolase protein 28precursor, putative, expressed
Contig4201_s_at	BF630713	4201	RCD1, putative, expressed
Contig9277_s_at		9277	Receptor-like kinase Xa21-binding protein 3

[a] Order of probe sets is identical to the deletion region in Figure 4 except Contig9277_s_at which is on another chromosome

[b] Unigene IDs were obtained from Barley HarvEST Assembly 21

[c] Annotations were obtained from PLEXdb (http://www.plexdb.org/index.php)

[d] Genes not present on the GeneChip®

Table 2. Segregation Analysis of F_2 Population

Cross (genotype)	*bcd1* X C.I.16151 (*Mla6, bcd1*) X (*Mla6, Bcd1*)		*bcd1* X Morex (*Mla6, bcd1*) X (*mla6, Bcd1*)			
Phenotype	Wild-type	Cell death	Resistance	Resistance + Cell death	Susceptible	Susceptible + Cell death
Observed	42	18	22	3	7	3
Expected	45	15	21	6	6	2
Model	3	1	9	3	3	1
X^2 = 0.56, *p*-value = 0.4543			X^2 = 2.21, *p*-value = 0.53			

Table 3. Primers for VIGS Constructs

Gene Name	Construct Name	Primer Name	Primer Sequence
Contig4201_s_at	BSMV:4201_1	VIGS4201f1	ATATTAATTAAGGAACATTTGATAGTGGCGTGG
		VIGS4201r1	TATGCGGCCGCTGTCAGAACGAGGAACTTTTGTGG
	BSMV:4201_2	VIGS4201f2	ATATTAATTAACCTCCCAATGCTTTTCGCTG
		VIGS4201r2	TATGCGGCCGCGGCTGCTCACAGATTACTGACTGG
Contig8225_at	BSMV:8225_1	VIGS8225f1	ATATTAATTAAAGCAACGGCAGCAGCAAATG
		VIGS8225r1	TATGCGGCCGCGCACTTGGCATACAGTTCTCCAG
	BSMV:8225_2	VIGS8225f2	ATATTAATTAAGCACAACAACTTCTCATCCTTCG
		VIGS8225r2	TATGCGGCCGCTTCAGCATCTCAATCTCTTCCTCC
Contig12722_s_at	BSMV:12722_1	VIGS12722f1	ATATTAATTAAGGAATCAGCAGGGAAGGCTTAG
		VIGS12722r1	TATGCGGCCGCTCCAAAACCTCACACTCCTACTACG
	BSMV:12722_2	VIGS12722f2	ATATTAATTAACGTTCGCTTCACTTCGTAGTAGG
		VIGS12722r2	TATGCGGCCGCAGCAAGGCAAAAATGGTGTCAG
CA031190	BSMV:CA031190_1	VIGS15423f1	ATATTAATTAAGGTTATGGGTGACGATGGTTCTC
		VIGS15423r1	TATGCGGCCGCTCCGTGGGTGATGGAAGTTATC
	BSMV:CA031190_2	VIGS15423f2	ATATTAATTAATACGAGATGACGCTCAAGAGGAC
		VIGS15423r2	TATGCGGCCGCTCCGTGGGTGATGGAAGTTATC
Contig24342_at	BSMV:24342_1	VIGS24342f1	ATATTAATTAATGTGACGCGGGTACGAGTAG
		VIGS24342r1	TATGCGGCCGCTCCAAGCTCTAGGTGGCAGT
	BSMV:24342_2	VIGS24342f2	ATATTAATTAAGGTTTGCACTTGCACATCAG
		VIGS24342r2	TATGCGGCCGCTGCATACAAAATCGAAGTCCA
BF267800	BSMV:BF267800_1	VIGS39836f1	ATATTAATTAACGTTGTTGTTGCTTTTCGCCTC
		VIGS39836r1	TATGCGGCCGCATGGTGGTAGTAGTGCTTGGAGATG
	BSMV:BF267800_2	VIGS39836f2	ATATTAATTAATCCAAGCACTACTACCACCATGGC
		VIGS39836r2	TATGCGGCCGCTTGCCGTACACGTTGGTCTGCATC

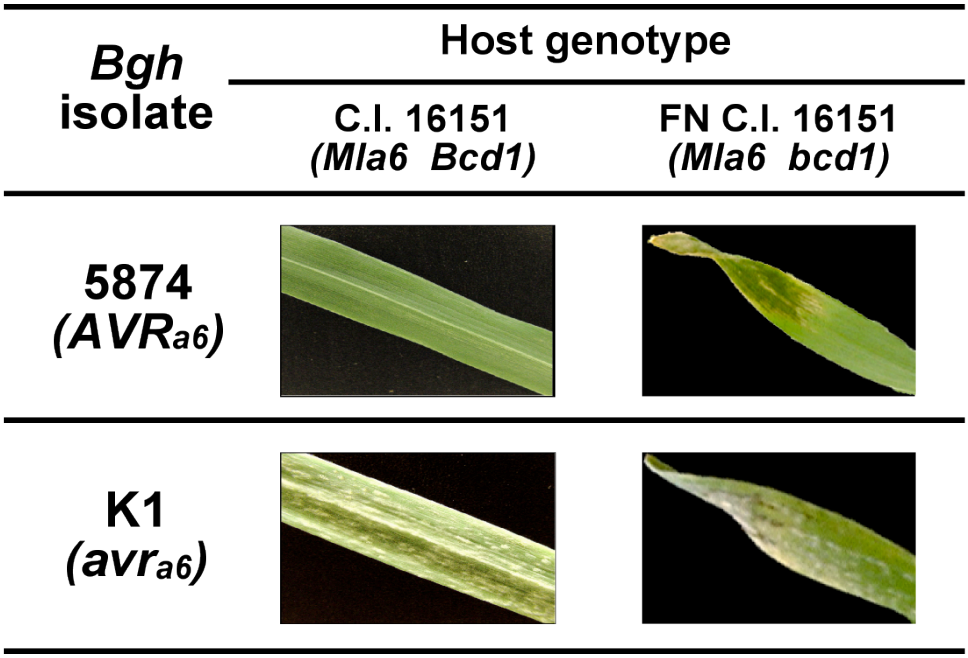

Figure 1. Experiment Design
The experiment design was based on a split-split-plot design with replications as blocks, *Bgh* isolate as the whole-plot factor, plant genotype as the split-plot factor, and time as the split-split-plot factor. Seven-day old plants (1st leaf) of wild-type (C.I. 16151) and fast-neutron derived mutant (FN C.I. 16151) were inoculated with respective *Bgh* isolates 5874 (AVR_{a6}) and K1 (avr_{a6}). Fifteen first leaves of inoculated barley seedlings were harvested at 0, 8, 16, 20, 24, and 32 hours after inoculation (hai). One Barley1 GeneChip was used for each of the 72 split-split-plot experiment units corresponding to 3 replications x 2 isolates x 2 genotypes x 6 time points. The infection types shown above were photographed 7 days after inoculation.

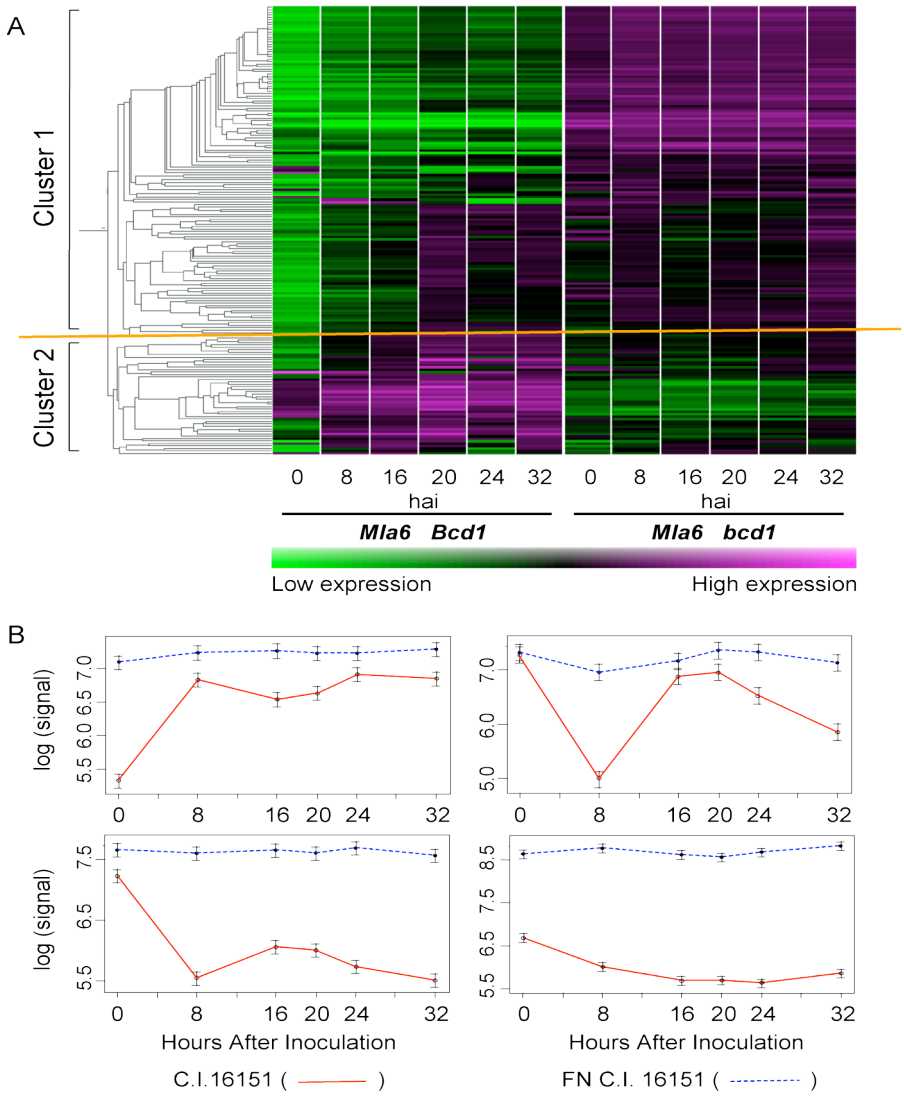

Figure 2. Expression Profiles of 182 Differentially Expressed Genes in Wild-type and Mutant upon *Bgh* Inoculation.

(A) Average signal intensities at each time point in the wild-type C.I. 16151 and mutant inoculated with *Bgh* 5874 and K1 were used in the cluster analysis. A data matrix was constructed with genes in rows and time points of genotype-average isolate combinations in columns. A Pearson correlation was used to measure similarities of transcript accumulation in a pairwise manner. Hierarchical clustering was performed using GeneSpring 5.1 software.

(B) Reciprocal expression profiles of representative genes in cluster 1. The natural logarithm of signal intensities in wild-type and mutant with the average of the two *Bgh* isolates were plotted in graphs. Standard errors were calculated based on three independent replications.

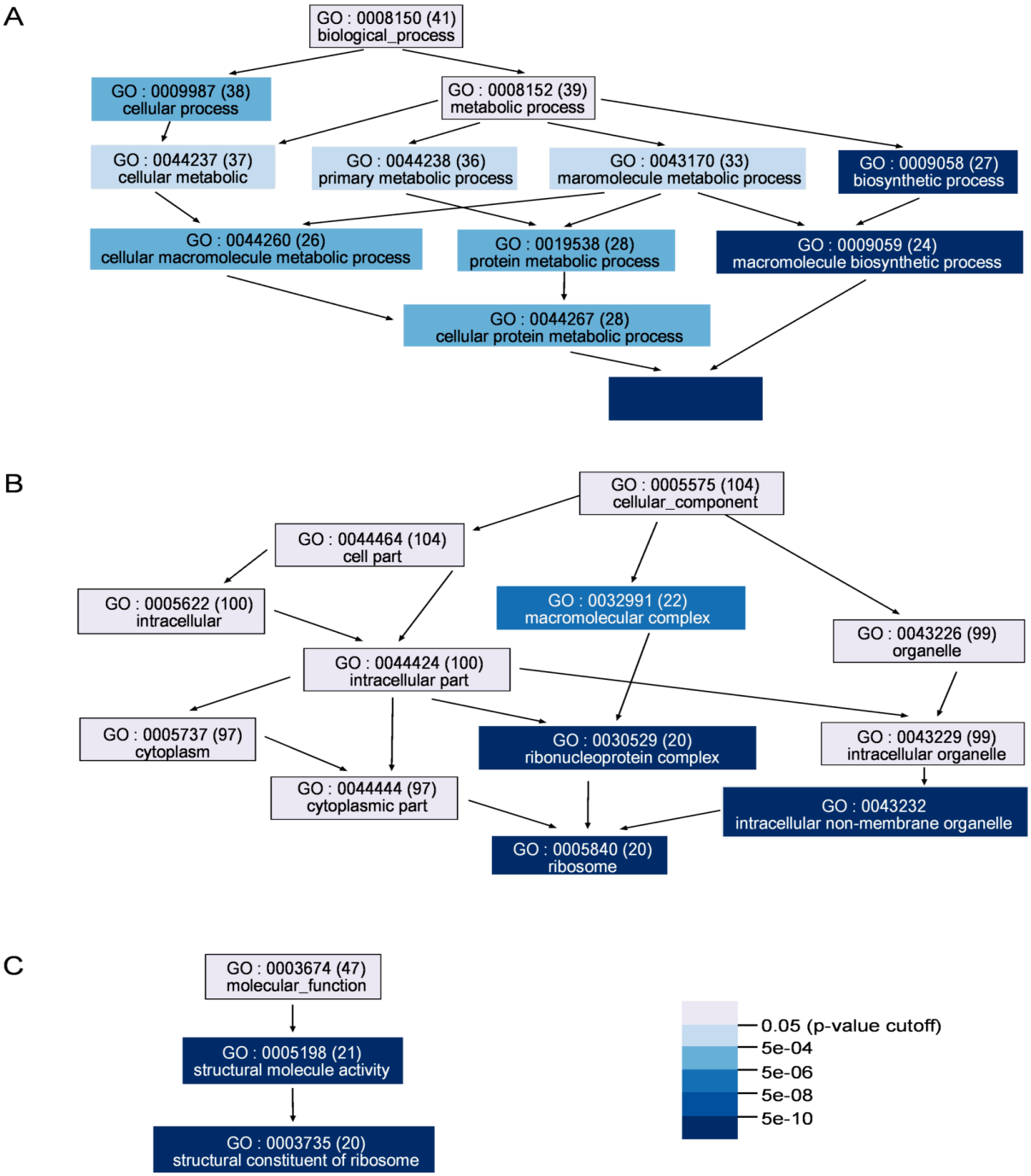

Figure 3. GO Analysis of Differentially Expressed Genes Between Wild-type and Mutant.

One hundred eight-two differentially expressed probsets were annotated using the Gene Ontology based functional enrichment analysis tool at EasyGO (http://bioinformatics.cau.edu.cn/easygo/) using the default settings. Images were modified from the output.

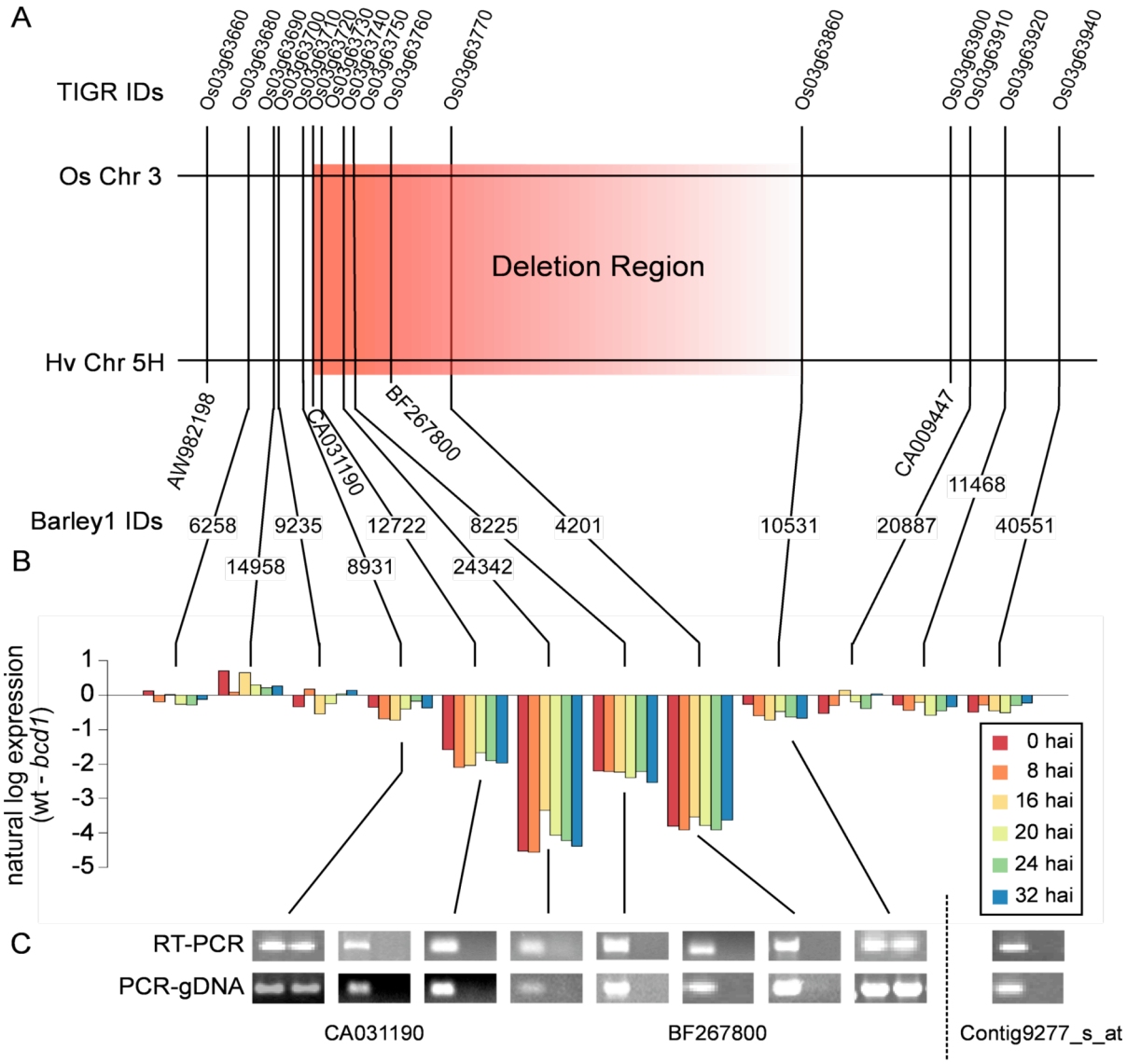

Figure 4. Integrated map depicting syntenous region in rice aligned with barley genetic map, PCR results, and expression data.

(A) Alignment of syntenous rice chromosome region (chromosome 3) with barley positional orthologs on chromosome 5H. Barley genes were ordered and positioned according to the rice synteny.

(B) Natural log expression difference of genes encompassing the deletion region between wild-type and *bcd1* for 0, 8, 16, 20, 24, and 32 hours after inoculation according to the transcript expression data. Numbers across each line between A and B are Barley1 IDs for each probset. Genes with GeneBank IDs are those not present on the GeneChip.

(C) Genomic-PCR and RT-PCR of deletion region genes, with wild-type C.I. 16151 on the right and mutant *bcd1* on the left of each gel. Contig9277_s_at were not mapped to this deletion region.

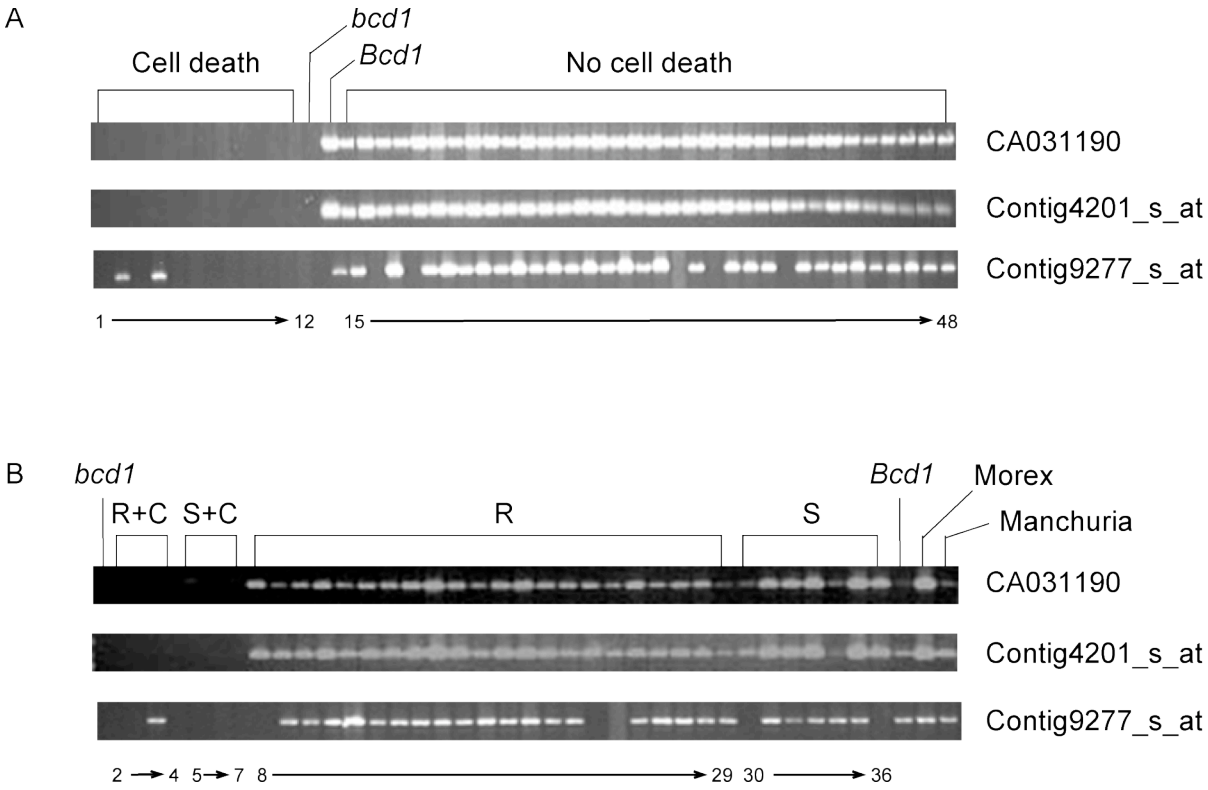

Figure 5. Amplification of *bcd1* deletion genes in F_2 Populations.

DNAs extracted from the F_2 population of **(A)** C.I. 16151 × *bcd1* and **(B)** F_2 population of Morex × *bcd1*, were used as templates, respectively. PCR amplification was conducted to detect deletion genes (Contig12722_s_at, Contig24342_at, Contig8225_at, Contig4201_s_at CA031190 and BF267800). Except Contig9277_s_at, all other 5 genes couldn't be amplified in cell death plants, but could be amplified in plants without cell death. R: resistant plants; S: susceptible plants; C: cell death plants. Other deleted genes showed the similar results of CA031190 and Contig4201_s_at as pictured above, and the deletion of them showed co-segregation with the cell death. Contig9277_s_at is the one that did not co-segregate with cell death.

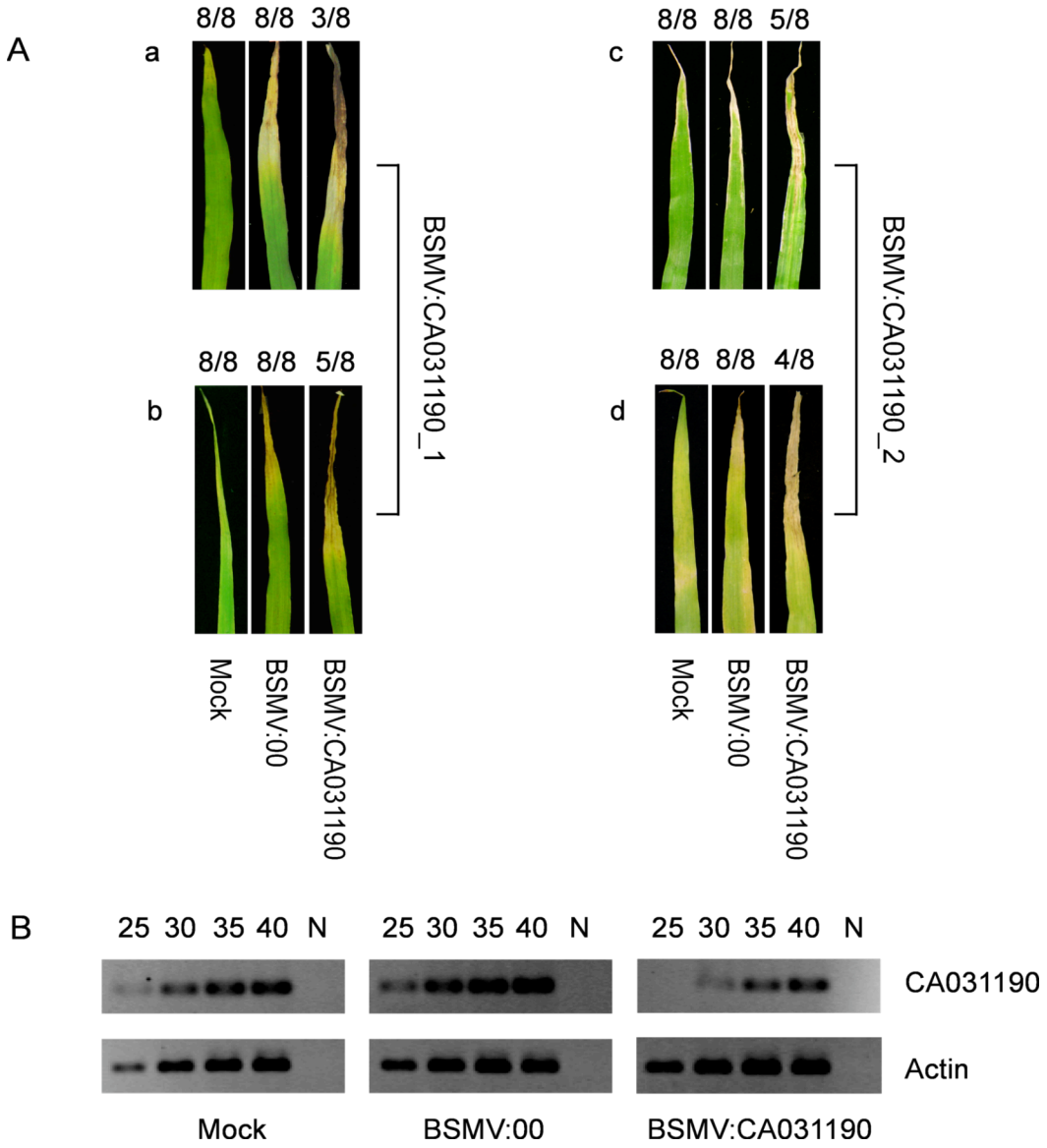

Figure 6. Silencing of CA031190 Caused the Tip Cell Death.

(A) Compared to the empty vector infected plants, plants infected with BSMV:CA031190 developed cell death equivalent to the *bcd1* mutant after *Bgh* 5874 inoculation. Eight plants were mechanically infected with each construct, and the number above each leaf shows the number of plants presenting the pictured phenotype. Each construct was used in four replicate experiments, of which two replicates (a,b and c,d) are pictured above. The two silencing constructs of CA031190 generated similar results.

(B) RT-PCR analysis showing the effect of VIGS on CA031190 transcript accumulation. Ethidium bromide-stained agarose gels visualized RT-PCR products. Lane N represents the no-template control, in which the RT-PCR mix without reverse transcriptase was used as template.

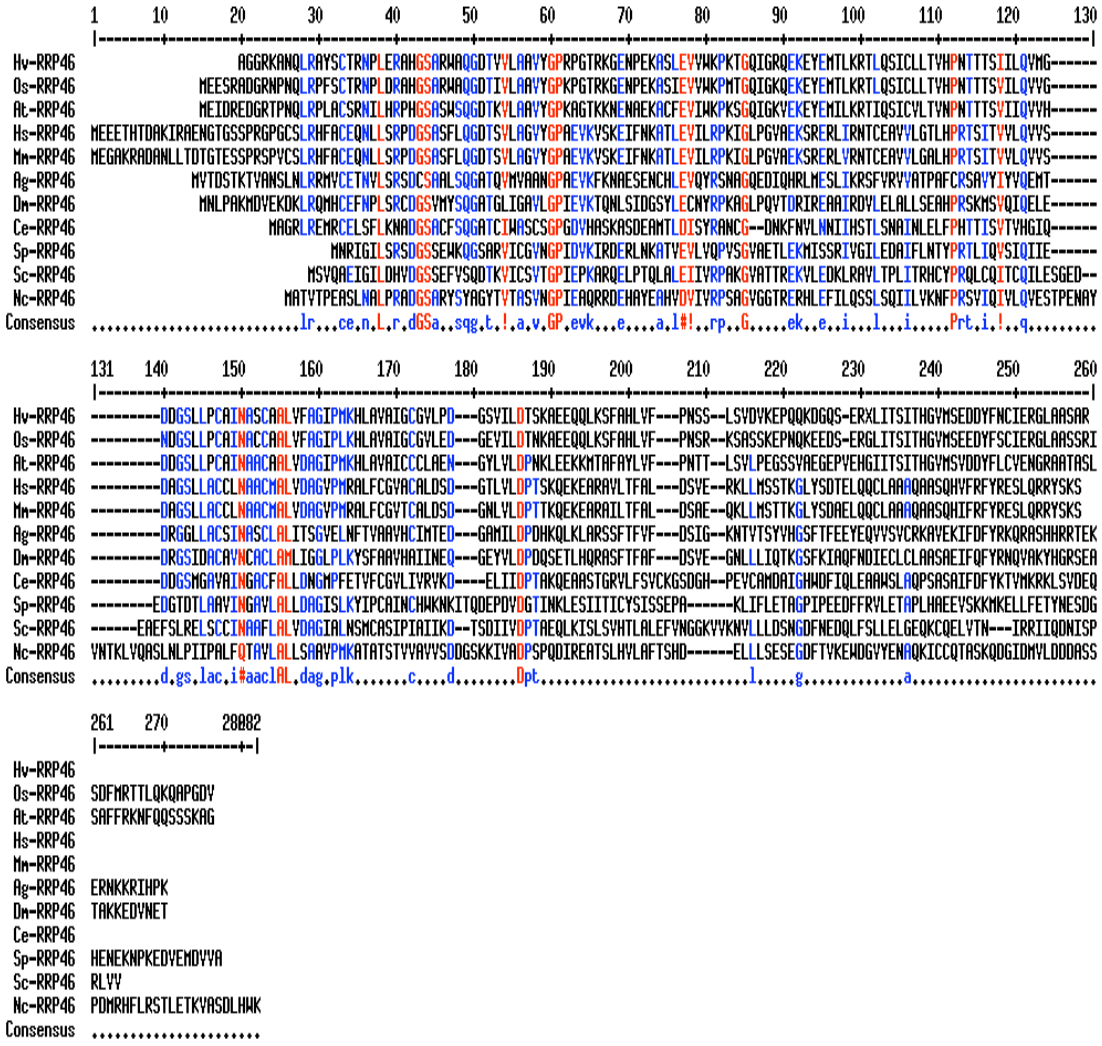

Figure 7. Cross-taxa Sequence Alignment of Eleven RRP46 Proteins.

Amino acid sequence alignment of 11 RRP46 proteins from plants (*H. vulgare, O. sativa, A. thaliana*), vertebrates (*H. sapiens, M. musculus*), nematode (*C. elegans*), fungi (*S. cerevisia, S. pombe, N. crassa*), and insects (*A. gambiae, D. melanogaste*r) was conducted using the Multiple sequence alignment located on http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html. The colored sequences represent the consensus among different species. The conserved RNase-PH domain is positioned from amino acid residue 28 to 187.

Supplemental Table 1. List of 182 Differentially Expressed Genes between *bcd1* and C.I. 16151 Over Time (*p* value < 0.001)

NO	Affymetrix probeset ID[a]	*p*-value	Predicted function [Species] E value[a]	GO Annotation		
				Biological process	**Cellular component**	**Molecular function**
1	baak12b08_s_at	6.96E-06	putative 60S ribosomal protein L38 [*Oryza sativa*] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity
2	Contig1040_at	3.04E-07	ubiquitin/ribosomal polyprotein [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	structural molecule activity
3	Contig10627_at	1.79E-07	mucin-like protein[*Oryza sativa*] 0		cell part organelle	
4	Contig11093_at	4.36E-09	putative snRNP protein [*Oryza sativa*] 0	cellular process physiological process	cell part membrane-enclosed lumen organelle organelle part protein complex	
5	Contig11187_at	2.34E-07	mitochondrial glycoprotein-like [*Oryza sativa*] 0		cell part membrane-enclosed lumen organelle organelle part	
6	Contig11344_at	2.32E-06	immunophilin / FKBP-type peptidyl-prolyl cis-trans isomerase -related [*Arabidopsis thaliana*] 2.00E-45	cellular process physiological process		catalytic activity binding
7	Contig11380_at	6.60E-07	Putative ribosomal protein S5 [*Oryza sativa*] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity
8	Contig11520_at	3.09E-07	single-strand DNA binding protein-like [*Oryza sativa*] 0		cell part organelle	binding

9	Contig1200_at	2.75E-06	elongation factor 1 gamma-like protein [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	translation regulator activity binding
10	Contig12434_at	6.60E-07	Cluster: 50S ribosomal protein L21 [*Oryza sativa*] 4.00E-66	cellular process physiological process	cell part organelle protein complex	binding structural molecule activity signal transducer activity
11	Contig13809_at	2.18E-06	putative nicotinate phosphoribosyltransferase [*Oryza sativa*] 2.00E-98		cell part organelle	
12	Contig13843_at	1.3E-05	exonuclease RRP41 [*Arabidopsis thaliana*] 0	cellular process physiological process		catalytic activity binding
13	Contig1453_x_at	5.90E-06	60S ribosomal protein L5-1 [*Oryza sativa*] 0	cellular process physiological process	cell part organelle protein complex membrane-enclosed lumen organelle part	binding
14	Contig14727_s_at	1.52E-06	putative pol polyprotein [*Oryza sativa*] 0	cellular process physiological process	cell part organelle	
15	Contig15436_at	2.23E-06	protein periodic tryptophan protein 2, putative, expressed [*Oryza sativa*] 2.00E-87			binding
16	Contig1561_at	7.28E-06	putative 60S ribosomal protein L38 [*Oryza sativa*] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity

17	Contig1601_at	7.45E-08	40S ribosomal protein S16 [*Oryza sativa*] 9.00E-74	cellular process physiological process	cell part organelle protein complex	structural molecule activity
18	Contig1623_at	1.08E-06	60S ribosomal protein L3 [*Oryza sativa*] 0	reproduction development cellular process physiological process	protein complex cell part organelle organelle part	structural molecule activity
19	Contig166_at	9.23E-07	60S ribosomal protein L14 (RPL14A) [*Arabidopsis thaliana*] 2.00E-54	cellular process physiological process	cell part organelle protein complex	structural molecule activity
20	Contig16629_s_at	6.1E-05	Hypothetical protein [*Oryza sativa*] 0			binding
21	Contig1696_at	3.47E-06	putative ribosomal protein L32 [*Oryza sativa*] 0	cellular process physiological process	cell part organelle protein complex	structural molecule activity
22	Contig1794_s_at	4.23E-06	Putative S-adenosylhomocystein hydrolase 2 [*Hordeum vulgare*] 0	cellular process physiological process regulation of biological process reproduction development		catalytic activity
23	Contig1818_at	1.54E-06	Ribosomal protein L11 [*Triticum aestivum*] 0	cellular process physiological process	protein complex cell part organelle	binding structural molecule activity
24	Contig18974_at	1.59E-06	thiF family protein [*Arabidopsis thaliana*] 0		cell part organelle	
25	Contig1913_s_at	6.91E-07	60S acidic ribosomal protein P0 [*Oryza sativa*] 2.00E-90	cellular process physiological process	protein complex cell part organelle	structural molecule activity

26	Contig1938_s_at	3.18E-06	ribosomal protein L15 [*Homo sapiens*] 0	cellular process physiological process	cell part organelle protein complex	structural molecule activity
27	Contig1949_s_at	6.83E-07	40Sribosomal protein S12 [*Hordeum vulgare*] 1.00E-63	cellular process physiological process	cell part protein complex organelle	structural molecule activity
28	Contig1978_at	5.04E-07	putative 40S ribosomal protein S5 [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	structural molecule activity
29	Contig2001_at	1.93E-06	60S ribosomal protein L7a [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	structural molecule activity
30	Contig20318_at	3.4E-05	OSJNBa0019D11.5 [*Oryza sativa*] 0	cellular process physiological process	cell part organelle organelle part	binding structural molecule activity
31	Contig2093_s_at	9.15E-07	40S ribosomal protein S23 [*Oryza sativa*] 0		cell part organelle	
32	Contig2094_s_at	1.57E-07	40S ribosomal protein S23 [*Oryza sativa*] 0		cell part organelle	
33	Contig2100_s_at	2.69E-06	60S ribosomal protein L9 [*Oryza sativa*] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity

34	Contig2103_at	3.12E-06	60S ribosomal protein L9 [Oryza sativa] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity
35	Contig2106_s_at	2.30E-06	60S ribosomal protein L9 [Oryza sativa] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity
36	Contig2342_at	2.67E-07	contains similarity to 40S ribosomal protein S17 [*Oryza sativa*] 0		cell part organelle	
37	Contig2345_s_at	7.11E-07	contains similarity to 40S ribosomal protein S17 [*Oryza sativa*] 0		cell part organelle	
38	Contig2448_s_at	2.42E-06	ribosomal protein L35A [*Zea mays*] 0	cellular process physiological process	cell part organelle protein complex	structural molecule activity
39	Contig2521_s_at	3.60E-07	40S ribosomal protein S15A (RPS15aA) [*Arabidopsis thaliana*] 6.00E-25	cellular process physiological process	protein complex cell part organelle organelle part	structural molecule activity
40	Contig2523_at	6.48E-06	40S ribosomal protein S15A (RPS15aA) [*Arabidopsis thaliana*] 6.00E-25	cellular process physiological process	cell part protein complex organelle	structural molecule activity
41	Contig2611_s_at	5.40E-06	putative 40S ribosomal protein S2 [*Oryza sativa*] 0	cellular process physiological process	cell part protein complex organelle organelle part	structural molecule activity

42	Contig2733_s_at	1.37E-06	60S ribosomal protein L37 (RPL37B) [*Arabidopsis thaliana*] 0	cellular process physiological process	cell part organelle protein complex	structural molecule activity
43	Contig3130_s_at	6.17E-06	putative prohibitin [*Oryza sativa*] 0	cellular process physiological process	cell part organelle	
44	Contig3262_at	1.64E-06	translational elongation factor Tu [*Oryza sativa*] 0	cellular process physiological process	cell part organelle	translation regulator activity binding
45	Contig3457_at	6.90E-06	probable fibrillarin [*Picea mariana*] 0	cellular process physiological process	cell part organelle	binding
46	Contig3457_x_at	6.90E-06	probable fibrillarin [*Picea mariana*] 0	cellular process physiological process	cell part organelle	binding
47	Contig373_s_at	1.40E-06	putative 40S ribosomal protein S15 [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	structural molecule activity
48	Contig3873_at	1.91E-06	LHY protein [*Phaseolus vulgaris*] 0		cell part organelle	binding
49	Contig3875_s_at	4.36E-06	MYB-related transcription factor (CCA1) [*Arabidopsis thaliana*] 1.00E-19		cell part organelle	binding
50	Contig397_x_at	6.57E-07	protein elongation factor 1-alpha, putative, expressed [*Oryza sativa*] 0	cellular process physiological process	cell part organelle	binding translation regulator activity
51	Contig4056_s_at	3.77E-06	pathogenesis-related protein 1A/1B precursor [*Hordeum vulgare*] 0	response to stimulus		

52	Contig4191_at	1.02E-08	putative nucleolar protein family A member 2 [*Oryza sativa*] 0	cellular process physiological process	cell part protein complex organelle	structural molecule activity
53	Contig429_s_at	3.51E-06	ribosomal S3Ae family, putative [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	structural molecule activity
54	Contig43_at	1.14E-06	putative 60S ribosomal protein L36 [*Oryza sativa*] 5.00E-52	cellular process physiological process	protein complex cell part organelle	structural molecule activity
55	Contig4654_at	7.28E-07	10 kDa chaperonin [*Oryza sativa* (japonica cultivar-group)] 0		cell part organelle	
56	Contig487_s_at	1.73E-07	putative nucleolin [*Oryza sativa*] 0		cell part organelle	
57	Contig5134_at	6.51E-07	mitochondrial chaperonin-60 [*Oryza sativa*] 0	cellular process physiological process response to stimulus	cell part organelle	binding
58	Contig5555_at	5.51E-07	putative 3-isopropylmalate dehydrogenase [*Oryza sativa*] 0	physiological process	cell part organelle	catalytic activity
59	Contig6438_s_at	2.06E-08	putative DNA-binding protein phosphatase 2C [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part	catalytic activity
60	Contig668_at	3.24E-07	protein elongation factor 1-alpha [*Oryza sativa*] 0	cellular process physiological process	cell part organelle	binding translation regulator activity

61	Contig722_at	2.84E-07	putative ribosomal protein L26 [*Oryza sativa*] 1.00E-52		cell part organelle	
62	Contig742_at	7.83E-08	putative nucleolin [Oryza sativa] 0			binding
63	Contig7549_at	6.45E-07	protein calmodulin-related protein 2, touch-induced, putative, expressed [*Arabidopsis thaliana*] 5.00E-66	cellular process physiological process	cell part	binding
64	Contig8747_at	2.50E-07	putative inosine monophosphate dehydrogenase [*Oryza sativa*] 0			catalytic activity
65	Contig9518_at	1.83E-06	HUELLENLOS-like protein [*Oryza sativa*] 0	cellular process physiological process	cell part organelle protein complex	structural molecule activity
66	Contig9815_at	4.96E-06	hypothetical protein OSJNBa0093M23.12 [*Oryza sativa*] 0	cellular process physiological process regulation of biological process	cell part organelle	transcription regulator activity
67	EBma05_SQ003_H21_at	7.49E-06	protein 60S ribosomal protein L37, putative, expressed [*Oryza sativa*] 4.00E-16	cellular process physiological process	cell part organelle protein complex	structural molecule activity
68	HA12A08u_s_at	7.06E-07	putative ribosomal protein S18 [*Triticum aestivum*] 1.00E-118	cellular process physiological process	protein complex cell part organelle organelle part	structural molecule activity
69	HB22P12r_x_at	3.90E-06	protein elongation factor 1-alpha, putative, expressed [*Oryza sativa*] 0	cellular process physiological process	cell part	binding translation regulator activity

70	HI05M04r_s_at	2.67E-07	60S ribosomal protein L5-1 [*Oryza sativa*] 0	cellular process physiological process	cell part organelle protein complex	structural molecule activity
71	HS18F06u_s_at	1.09E-06	60S ribosomal protein L7a [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle	structural molecule activity
72	HV12N24u_s_at	1.33E-06	putative GAR1 protein [*Arabidopsis thaliana*] 0		cell part organelle	
73	HY09M22u_s_at	1.33E-06	40S putative ribosomal protein S19 [*Oryza sativa*] 1.00E-160	cellular process physiological process	cell part organelle	binding
74	HZ45H16r_s_at	7.3E-05	prohibitin [*Zea mays*] 0	cellular process physiological process	cell part organelle	
75	rbasd18a22_s_at	1.80E-08	ribosomal protein S4 [*Oryza sativa*] 0	cellular process physiological process	protein complex cell part organelle organelle part	structural molecule activity
76	Contig1023_at	9.37E-07	guanine nucleotide-binding protein beta subunit-like protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
77	Contig1024_at	2.32E-06	40S ribosomal protein S8 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
78	Contig1038_x_at	3.93E-08	protein 40S ribosomal protein S27a, putative, expressed [*Oryza sativa*] 2.00E-64	no annotation	no annotation	no annotation
79	Contig10436_at	1.9E-05	VIP1 protein [*Avena fatua*] 0	no annotation	no annotation	no annotation

80	Contig107_s_at	3.45E-07	homologue to UP|Q6YLX4 (Q6YLX4) L41 ribosomal protein (Ribosomal protein L41) [*Hordeum vulgare*] 0	no annotation	no annotation	no annotation
81	Contig11429_at	1.21E-07	nrap protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
82	Contig11452_at	3.13E-07	U3 snoRNP-associated-related protein [*Arabidopsis thaliana*] 4E-13	no annotation	no annotation	no annotation
83	Contig12486_at	2.3E-05	unknown	no annotation	no annotation	no annotation
84	Contig1473_s_at	8.70E-08	60s ribosomal protein L21 [*Triticum aestivum*] 0	no annotation	no annotation	no annotation
85	Contig1474_at	3.40E-07	60S ribosomal protein L21 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
86	Contig1476_at	6.85E-06	60S ribosomal protein L21 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
87	Contig1487_at	4.25E-06	ribosomal protein P1 [*Triticum aestivum*] 1.00E-141	no annotation	no annotation	no annotation
88	Contig14871_at	4.47E-06	unknown	no annotation	no annotation	no annotation
89	Contig15079_at	3.25E-06	ribosomal protein L5 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
90	Contig15126_at	3.18E-06	expressed protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
91	Contig15467_at	7.04E-06	unknown	no annotation	no annotation	no annotation
92	Contig1550_s_at	1.1E-05	ribosomal protein s6 RPS6-2 [*Zea mays*] 0	no annotation	no annotation	no annotation
93	Contig16460_at	7.86E-06	unknown	no annotation	no annotation	no annotation
94	Contig16498_at	3.03E-10	unknown	no annotation	no annotation	no annotation
95	Contig17521_at	3.03E-06	none	no annotation	no annotation	no annotation

96	Contig1809_at	7.67E-08	acidic ribosomal protein P2 [*Triticum aestivum*] 0	no annotation	no annotation	no annotation
97	Contig1810_at	2.36E-07	40S ribosomal protein S9 (RPS9C) [*Arabidopsis thaliana*] 6.00E-96	no annotation	no annotation	no annotation
98	Contig18608_at	5.47E-06	OSJNBa0066C06.6 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
99	Contig187_at	8.15E-06	OSJNBa0029H02.21 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
100	Contig1897_s_at	4.21E-08	none	no annotation	no annotation	no annotation
101	Contig1936_at	1.78E-06	ribosomal protein L15 [Homo sapiens] 0	no annotation	no annotation	no annotation
102	Contig21068_at	1.6E-05	none	no annotation	no annotation	no annotation
103	Contig2136_at	4.21E-06	elongation factor 1-beta [*Triticum aestivum*] 0	no annotation	no annotation	no annotation
104	Contig2301_at	1.95E-08	putative 60S ribosomal protein L12 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
105	Contig2341_x_at	6.05E-07	putative ribosomal protein L27 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
106	Contig2560_at	1.48E-06	none	no annotation	no annotation	no annotation
107	Contig26243_s_at	2.75E-07	expressed protein [*Arabidopsis thaliana*] 0	no annotation	no annotation	no annotation
108	Contig2627_at	6.27E-06	40S ribosomal protein S14 [*Lupinus luteus*] 0	no annotation	no annotation	no annotation
109	Contig3123_at	1.86E-06	nucleosome/chromatin assembly factor A [*Zea mays*] 0	no annotation	no annotation	no annotation
110	Contig3314_at	5.57E-06	early drought induced protein [*Oryza sativa*] 4E-09	no annotation	no annotation	no annotation

111	Contig3403_s_at	4.40E-06	rpS28 [*Hordeum vulgare*] 6.00E-22	no annotation	no annotation	no annotation
112	Contig367_s_at	4.04E-06	60S ribosomal protein L36 (RPL36C) [*Arabidopsis thaliana*] 1.00E-147	no annotation	no annotation	no annotation
113	Contig3759_s_at	7.76E-09	nucleolar protein [*Arabidopsis thaliana*] 0	no annotation	no annotation	no annotation
114	Contig409_s_at	4.27E-07	cold shock protein-1 [*Triticum aestivum*] 8.00E-22	no annotation	no annotation	no annotation
115	Contig5232_at	7.68E-07	putative ribosomal protein L34 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
116	Contig664_x_at	1.31E-08	homologue to UP|Q75QN8 (Q75QN8) Cold shock domain protein 3 [*Hordeum vulgare*] 0	no annotation	no annotation	no annotation
117	Contig692_s_at	7.67E-08	60S ribosomal protein L2 [*Arabidopsis thaliana*] 0	no annotation	no annotation	no annotation
118	Contig9026_at	1.36E-06	La related protein-like [*Oryza sativa*] 0	no annotation	no annotation	no annotation
119	EBed02_SQ003_C14_s_at	8.39E-07	40S ribosomal protein S8 [*Oryza sativa*] 3.00E-48	no annotation	no annotation	no annotation
120	EBem08_SQ002_L02_s_at	4.79E-06	60S ribosomal protein L5-1 [*Oryza sativa*] 1.00E-111	no annotation	no annotation	no annotation
121	EBem08_SQ004_N04_at	4.13E-06	weakly similar to UP|Q945S1 (Q945S1) U2 auxiliary factor small subunit1[*Hordeum vulgare*] 1.00E-149	no annotation	no annotation	no annotation
122	EBem09_SQ001_B21_s_at	1.78E-07	putative ribosomal protein S4 [*Oryza sativa*] 7.00E-97	no annotation	no annotation	no annotation
123	EBem09_SQ005_B14_s_at	8.47E-06	similar to UP|Q9FT78 (Q9FT78) P23 co-chaperone [*Hordeum vulgare*] 1E-104	no annotation	no annotation	no annotation

124	EBem10_SQ004_B20_s_at	3.73E-06	homologue to UP|Q94GV7 (Q94GV7) Cytoplasmic ribosomal protein L18 [*Hordeum vulgare*] 2E-85	no annotation	no annotation	no annotation
125	EBem10_SQ004_P21_x_at	2.54E-08	similar to UP|Q9XEA7 (Q9XEA7) Cysteine synthase [*Hordeum vulgare*] 4E-59	no annotation	no annotation	no annotation
126	EBma03_SQ003_N08_s_at	3.16E-08	putative nucleolar protein family A member 2 [*Oryza sativa*] 9E-42	no annotation	no annotation	no annotation
127	EBpi03_SQ003_A03_s_at	3.92E-06	ribosomal protein L17 [*Arabidopsis thaliana*] 4E-12	no annotation	no annotation	no annotation
128	EBro08_SQ002_I04_x_at	1.91E-06	hypothetical protein [*Oryza sativa*] 1E-08	no annotation	no annotation	no annotation
129	HA03D07u_s_at	8.88E-07	60S ribosomal protein L17-1 [*Hordeum vulgare*] 2.00E-91	no annotation	no annotation	no annotation
130	HA11C13u_s_at	7.74E-07	putative transcription factor [*Oryza sativa*] 3.00E-88	no annotation	no annotation	no annotation
131	HA27E10r_x_at	6.63E-06	60S ribosomal protein L35 [*Euphorbia esula*] 1.00E-130	no annotation	no annotation	no annotation
132	HA30O17r_s_at	8.49E-06	40S ribosomal protein S29 (RPS29A) [*Arabidopsis thaliana*] 5.00E-24	no annotation	no annotation	no annotation
133	HB30J05r_at	3.85E-08	unknown	no annotation	no annotation	no annotation
134	HD04N07u_at	9.40E-07	unknown	no annotation	no annotation	no annotation
135	HI05C01u_s_at	9.39E-06	putative 60S ribosomal protein L5 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
136	HS09O14r_s_at	4.92E-06	homologue to UP|Q75QN8 (Q75QN8) Cold shock domain protein 3 [*Hordeum vulgare*] 2E-86	no annotation	no annotation	no annotation

137	HU08M20u_x_at	1.18E-06	60S ribosomal protein L5-1 [Oryza sativa] 1.00E-76	no annotation	no annotation	no annotation
138	HU08O12u_s_at	2.92E-08	putative nucleolin [*Oryza sativa*] 0	no annotation	no annotation	no annotation
139	HV14N24u_s_at	6.42E-08	putative 40S ribosomal protein 25S [*Oryza sativa*] 1.00E-117	no annotation	no annotation	no annotation
140	HVSMEb0004N24r2_at	3.11E-06	similar to UP|O81126 (O81126) 9G8-like SR protein (RSZp22 splicing factor) [*Hordeum vulgare*] 0	no annotation	no annotation	no annotation
141	HW02F22u_s_at	4.74E-07	ribosomal protein L15 [*Homo sapiens*] 0	no annotation	no annotation	no annotation
142	HY05A23u_at	7.99E-07	none	no annotation	no annotation	no annotation
143	HY09G23u_s_at	8.01E-08	elongation factor 1-beta [*Triticum aestivum*] 0	no annotation	no annotation	no annotation
144	HZ51D22r_s_at	6.84E-06	B1358B12.15 protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
145	rbags12n24_s_at	3.60E-06	weakly similar to UP|O04697 (O04697) DNA-binding protein PD2 [*Hordeum vulgare*] 1.00E-156	no annotation	no annotation	no annotation
146	rbags18e07_s_at	8.06E-08	guanine nucleotide-binding protein beta subunit-like protein [*Oryza sativa*] 1.00E-112	no annotation	no annotation	no annotation
147	S0001100150B08F1_s_at	3.09E-06	putative phosphoenolpyruvate/phosphate translocator [*Oryza sativa*] 1.00E-15	no annotation	no annotation	no annotation
148	Contig12567_at	7.34E-06	cinnamoyl-CoA reductase family [*Arabidopsis thaliana*] 2.00E-21	cellular process physiological process	cell part	catalytic activity
149	Contig12842_at	1.2E-05	glycosyl hydrolase family 3 [*Arabidopsis thaliana*] 9.00E-44	cellular process physiological process	cell part organelle	

150	Contig13884_at	1.38E-06	RRM-containing protein [*Arabidopsis thaliana*] 0		cell part organelle	binding
151	Contig14052_at	5.96E-07	putative calmodulin binding transporter protein [*Hordeum vulgare*] 0	cellular process physiological process	cell part	transporter activity binding
152	Contig15796_at	4.13E-08	p34cdc2 [*Triticum aestivum*] 0	cellular process physiological process		catalytic activity binding
153	Contig16143_at	1.1E-05	hypothetical protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
154	Contig16309_at	0.00031	putative dihydroorotase [*Oryza sativa*] 0	cellular process physiological process		catalytic activity
155	Contig16393_at	3.33E-07	UMP synthase [*Oryza sativa*] 0	cellular process physiological process	cell part organelle	catalytic activity
156	Contig19139_at	2.08E-06	OSJNBa0013K16.11 protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
157	Contig26351_at	5.10E-06	putative thioredoxin m2 [*Pisum sativum*] 6.00E-25	no annotation	no annotation	no annotation
158	Contig3928_s_at	3.19E-06	putative cinnamyl-alcohol dehydrogenase [*Oryza sativa*] 0	no annotation	no annotation	no annotation
159	Contig393_at	1.73E-06	alcohol dehydrogenase 3 [*Hordeum vulgare*] 0	cellular process physiological process response to stimulus		catalytic activity
160	Contig4021_at	4.8E-05	OSJNBa0060D06.16 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
161	Contig4280_s_at	1.2E-05	hypothetical protein OJ1714_H10.110 [*Oryza sativa*] 0		cell part organelle	

162	Contig4499_s_at	7.48E-06	tetratricopeptide repeat (TPR)-containing protein [*Arabidopsis thaliana*] 0	no annotation	no annotation	no annotation
163	Contig4983_s_at	1.1E-05	putative ribosomal protein [*Oryza sativa*] 1.00E-168	no annotation	no annotation	no annotation
164	Contig5003_at		ATP-dependent Clp protease proteolytic subunit (ClpP5) [*Arabidopsis thaliana*] 0		cell part organelle	
165	Contig5045_s_at	2.64E-06	hypotheticzl protein [*Oryza sativa*] 9E-29	no annotation	no annotation	no annotation
166	Contig5420_at	8.96E-06	serine carboxypeptidase II-1 precursor (EC 3.4.16.6) (CP-MII.1) [*Hordeum vulgare*] 0	cellular process physiological process		catalytic activity
167	Contig5817_at	3.16E-07	similar to methionyl-tRNA synthetase [*Oryza sativa*] 1.00E-167	cellular process physiological process	cell part organelle	binding
168	Contig6674_at	5.66E-07	serine carboxypeptidase II-2 precursor [*Hordeum vulgare*] 0	no annotation	no annotation	no annotation
169	Contig6830_at	2.47E-06	phosphoserine phosphatase SerB, putative [*Oryza sativa*] 0		cell part organelle	
170	Contig6882_at	5E-05	probable ubiquitin activating enzyme 2 [*Picea mariana*] 0	no annotation	no annotation	no annotation
171	Contig6889_at	1.68E-06	putative ML domain protein [*Oryza sativa*] 0		cell part	
172	Contig8292_at	4.56E-06	hypothetical protein OJ1741_B01.8 [Oryza sativa] 0		cell part organelle	
173	Contig8375_at	6.49E-07	hypothetical protein [*Oryza sativa*] 0		cell part	

174	Contig9681_at	6.7E-05	putative peptide transporter [*Oryza sativa*] 0	cellular process physiological process	cell part	transporter activity
175	EBpi01_SQ002_N03_at	1.4E-05	unknown	no annotation	no annotation	no annotation
176	HW02G06T_s_at	0.00017	OSJNBa0095E20.4 protein [*Oryza sativa*] 0	no annotation	no annotation	no annotation
177	Contig10984_at	2.13E-07	P0501G01.24 [*Oryza sativa*] 0	no annotation	no annotation	no annotation
178	Contig11021_at	7.11E-06	putative monoterpene synthase [*Oryza sativa*] 0	physiological process	cell part	catalytic activity
179	Contig13547_at	1.1E-05	isoamylase-type starch debranching enzyme ISO2 [*Zea mays*] 0	no annotation	no annotation	no annotation
180	Contig13774_at	7.7E-05	hypothetical protein [*Oryza sativa*] 0		cell part organelle	
181	Contig19651_at	8.22E-06	F-box protein family [*Arabidopsis thaliana*] 3.00E-76		cell part organelle	
182	Contig9978_at	9.85E-08	protein mitochondrial glycoprotein, expressed [*Oryza sativa*] 1.00E-78		cell part membrane-enclosed lumen organelle organelle part	

#1-147 represent genes whose transcript accumulation is constitutively higher in the *bcd1* mutant as compared to wild-type;
#148-176 represent genes whose transcript accumulation is lower than wild-type;
#177-182 represent genes with variable patterns of transcript accumulation.
[a] BLAST e-value match score.

GO reference numbers for each category.

Biological process		**Cellular component**		**Molecular function**	
GO:0009987	cellular process	GO:0044464	cell part	GO:0005198	structural molecule activity
GO:0007582	physiological process	GO:0043234	protein complex	GO:0003824	catalytic activity
GO:0000003	reproduction	GO:0043226	organelle	GO:0005488	binding
GO:0007275	development	GO:0031974	membrane-enclosed lumen	GO:0045182	translation regulator activity
GO:0050789	regulation of biological process	GO:0044422	organelle part	GO:0004871	signal transducer activity
GO:0050896	response to stimulus			GO:0005215	transporter activity
				GO:0030528	transcription regulator activity

Supplemental Table 2. PCR Primers for Candidate Deletion Genes

Probeset Name	Gene Name	Primer Name	Primer Sequence
Contig4201_s_at	Hv21_4200[a]	PCR4200f	GTAAGGGACGAGATGATGCT
		PCR4200r	GAGATCACATGAGCCAACTC
Contig4201_s_at	Hv21_4201[a]	PCR4201f	GATAGTGGCGTGGATGATCT
		PCR4201r	GGTACATCACGGTACAAGCTG
Contig4424_at	Hv21_4424[a]	PCR4424f	TACACTGGTTCTGGTCTATCTG
		PCR4424r	CGACTCACTGATGAAGTCTCTT
Contig7850_at	Hv21_7850[a]	PCR7850f	CGCTATGTTGAAGGAGTCTGAG
		PCR7850r	AATAGCAGCTAGAGGCACTG
Contig8225_at	Hv21_8225[a]	PCR8225f	GAGAGGACAGAAACATCTCCTG
		PCR8225r	AGTACCTCACCCTCTATGGTTC
Contig8931_at	Hv21_8931	PCR8931f	CTAACCAAACCTCCACCCCTAC
		PCR8931r	GCCTCCTTTTGTTCTCTGTCTG
Contig9277_s_at	Hv21_9277[a]	PCR9277f	GGAGAAGAAGATCTTGAAGGAC
		PCR9277r	GATCAGATCTGTGCATCATTC
Contig10531_at	Hv21_10531	PCR10531f	CGAGGTAGGAAGGAAGGAAG
		PCR10531r	GACTCGGTCACACGACAAG
Contig10758_at	Hv21_10758[a]	PCR10758f	TCACTAGTAAAGGCTCAAGCTC
		PCR10758r	CTCGGTTTTATGTACACAGACG
Contig10761_at	Hv21_10761[a]	PCR10761f	CTCCGATTGTAAAGATGAGG
		PCR10761r	AGTCTGTAGCGTGGCATATAG
Contig11646_at	Hv21_11646[a]	PCR11646f	CTGCAAGGTCTACGAGATTC
		PCR11646r	ATCAGCCTGTCAGAAATCAG
Contig12722_s_at	Hv21_12722[a]	PCR12722f	CAGGGAAGGCTTAGTGAGAC
		PCR12722f	CATCTTGACATTAGCACAGG
Contig12739_at	Hv21_12739[a]	PCR12739f	CTAGCAACGTTACGAGAAGTG
		PCR12739r	CACAACCCAGTTACGAAGTTAC
Contig14176_at	Hv21_14176[a]	PCR14176f	ATCTGCAGTTGATAAGGTTGAC
		PCR14176r	TGCATCGCAGAGTCTAAAAC
	Hv21_15423[b]	PCR15423f	GGAGAAGGAGTACGAGATGAC
		PCR15423r	GACCCCTCTCTATACAGTTGAA
Contig17844_at	Hv21_17844[a]	PCR17844f	GATCAGGCGAGATTTACCAC
		PCR17844r	TACTCACCACACAAGTGCAG
Contig18852_at	Hv21_18852[a]	PCR18852f	AGATGGACGAGGAACTATTCT
		PCR18852r	CACTTGAATTAAGCTCTAGGAC
Contig23209_at	Hv21_23209[a]	PCR23209f	TACTTTCCTGTCAAACAGTTCC
		PCR23209r	GGGGTGTACAAAAATATACGTG
	Hv21_23851[b]	PCR23851f	CACTTGACCACCCAAACCTA
		PCR23851r	GGGAGGAAAGAACAAACAGA
Contig24342_at	Hv21_24342[a]	PCR24342f	CGGGTACGAGTAGATCGTTCA
		PCR24342r	TGCATACAAAATCGAAGTCC

Contig26136_at	Hv21_26136[a]	PCR26236f	CAGTACTGCACCTACTTCGAC
		PCR26236r	TGCTCATTATTCCTCCACTC
EBem08_SQ003_O18_at	Hv21_29269[a]	PCR29269f	AGGTGGAGATGGATCTGAAG
		PCR29269r	GGACTGTACTCGACTCAACTG
HK04F07r_at	Hv21_35010[a]	PCR35010f	TTTTGCATACACGAGCAGTT
		PCR35010r	CTTCCATGTTACATCGTGTG
HU07O24r_s_at	Hv21_38295[a]	PCR38295f	ACTAGACTAGAAGGGGGTCTGG
		PCR38295r	CTTCATCCTCATCTACAACG
HU07O24r_s_at	Hv21_38295[a]	PCR38295f2	TGATTAGTTCTGGAGGCTAACT
		PCR38295r2	TCCTCATCTACAACGTATCTCA
HV_CEa0006M06r2_s_at	Hv21_39578	PCR39578f	CCCAGCACCCTCACCTTC
		PCR39578r	TATTATTGGGGCCCTCGAAAG
	Hv21_39836[b]	PCR39836f	CGACGTTGTTGTTGCTTTTC
		PCR39836r	GTACACGTTGGTCTGCATCC
	Hv21_40653[b]	PCR40653f	GTTTACGACACCGCCAAG
		PCR40653r	GACTCGGTCACACGACAAG
	Hv21_41352[b]	PCR41352f	AGCTGGACTTCGAGTTCCTC
		PCR41352r	CCGACCTACGAAGAAGTTTT
M58754_at	Hv21_49400[a]	PCR49400f	CGAATTCACTCTCTTTCTCC
		PCR49400r	AGTTGCCTTGTACCCACATA
	Hv21_49617[b]	PCR49617f	CACCTCTTGGGGTAACTTTTAG
		PCR49617r	GTAGTTCTACGGATCTCCAATC

[a] 22 Candidate deleted genes identified in transcriptome analysis

[b] Genes not present on the GeneChip but identified around the deletion region according to rice synteny

CHAPTER 3. GENERAL CONCLUSIONS

GENERAL DISCUSSION

Programmed cell death is an important process in plants, serving essential roles in development and defense. In plant-pathogen interactions, cell death can occur in both compatible and incompatible responses. Moreover, defense related cell death is associate with plant development, involving 'cross-talk' between different signaling pathways (Greenberg, 1996; Pennell and Lamb, 1997; Heath, 1998; Greenberg and Yao, 2004). Identification of key factors that associate in these pathways helps understanding the complex regulation during the signaling transduction network among them. The work in this thesis presents the identification and isolation of a novel gene *Bcd1*, a regulator of ribosomal RNA processing, mediates an *R*-gene-independent cell death in barley-powdery mildew interactions. The abundance of ribosomal genes present in the mutant agreed with the ribosomal RNA processing function of *Bcd1*, suggesting the possible regulatory role of exosome in cell death development. The absence of *Bcd1* may cause the failure of normal ribosomal RNA processing, generating excessive rRNA and disorganized metabolism. These undermine the ribosomal RNA/protein signaling pathway, compromising the plants' normal metabolic and physiological process, thus, the plant becomes defenseless and prone to develop cell death under stress. In addition, the common cell death occurring in both compatible and incompatible responses suggests the common cell death pathway underlying the plant-pathogen interactions.

RECOMMENDATION FOR FUTURE RESEARCH

Further investigations can be performed as following:

1) Use Rapid Amplification of cDNA Ends (RACE) or BAC library hybridization to get the full length cDNA and full length clone.
2) Use Targeted Induced Local Lesions IN Genome (TILLING) to get the *bcd1* mutant. This single gene mutant can be used in overexpression experiment, where *Bcd1* gene is overexpressed to see if it can rescue the cell death phenotype.
3) Express *Bcd1* gene (or rice ortholog) in yeast RRP46 mutant to see if it complements the mutation.
4) Find the effectors of *Bcd1* and establish the detailed linkage between *Bcd1* and cell death progress.
5) Conduct time-course histological analysis to define the stages of cell death advancing during pathogen invasion.

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APPENDIX. ADDITIONAL DATA

This appendix contains the VIGS experiment data.

Appendix Table 1. Number of Plants in Each Category of VIGS Experiment

Construct	Rep1			Rep2		
	BSMV infected	cell death	no cell death	BSMV infected	cell death	no cell death
BSMV:12722_1	7	0	7	8	0	8
BSMV:12722_2	7	0	7	6	0	6
BSMV:24342_1	5	0	5	8	0	8
BSMV:24342_2	8	0	8	7	0	7
BSMV:4201_1	8	0	8	8	0	8
BSMV:4201_2	8	0	8	7	0	7
BSMV:8225_1	6	0	6	7	0	7
BSMV:8225_2	8	0	8	7	0	7
BSMV:BF267800_1	7	0	7	6	0	6
BSMV:BF267800_2	8	0	8	7	0	7
BSMV:CA031190_1	7	3	4	6	5	1
BSMV:CA031190_2	8	5	3	7	4	3
BSMV:00	8	0	8	8	0	8
Mock	0	0	8	0	0	8

Construct	Rep3			Rep4		
	BSMV infected	cell death	no cell death	BSMV infected	cell death	no cell death
BSMV:12722_1	6	0	6	8	0	8
BSMV:12722_2	6	0	6	7	0	7
BSMV:24342_1	7	0	7	8	0	8
BSMV:24342_2	5	0	5	6	0	6
BSMV:4201_1	8	0	8	8	0	8
BSMV:4201_2	7	0	7	8	0	8
BSMV:8225_1	7	0	7	8	0	8
BSMV:8225_2	6	0	6	6	0	6
BSMV:BF267800_1	5	0	5	5	0	5
BSMV:BF267800_2	7	0	7	8	0	8
BSMV:CA031190_1	8	4	4	7	3	4
BSMV:CA031190_2	5	2	3	6	3	5
BSMV:00	7	0	7	8	0	8
Mock	0	0	8	0	0	8

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